

T H E S I S

on

THE EXPERIMENTAL PATHOLOGY OF RENAL HYPERTENSION

submitted

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by

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## P R E F A C E.

The purpose of this research was the experimental study of renal hypertension. Volume I. commences with a review of the available methods of making repeated observations of arterial blood pressure in animals. There then follows a report of personal experience of these methods with special reference to the Carotid Loop Method of Van Leersum. The Volume concludes with a review of the "pressor body" hypothesis of hypertension.

The first portion of Volume II. is devoted to a review of experimental methods of producing vascular hypertension. Experimental oxalate nephritis is then described and is followed by an account of the hypertension that is found to be associated with it. The experimental investigation of the part played by the renal nerves in the production of this hypertension is then described.

The production of glomerulo-nephritis by nephrotoxic serum is described. The part played by the renal nerves in the hypertension of this nephritis is then discussed and experimental research into the problem described.

The/

The volume concludes with a summary and a brief discussion of the significance of the results.

During the early stages of this work it was found that the technical methods were beyond the scope of one individual and I have been extremely fortunate in having the able co-operation of Drs. Robert Kellar and Douglas Matthew. These workers are using the same methods to investigate allied problems in the toxæmias of pregnancy. They hope in the near future to present theses embodying their results.

I take this opportunity of expressing my gratitude to Professor Murray Drennan for his never failing interest, advice, and the hospitality of his Department. I am also greatly indebted to Professors Sir David Wilkie, I. de B. Daly, Melville Dunlop, T.J. Mackie, F.A.E. Crew, and Dr. C.P. Stewart for their continued interest and the facilities of their respective departments.

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For/





## C O N T E N T S.

### VOLUME 1.

	<u>Page</u>
Review of methods of measuring blood pressure in animals. ....	1.
Personal experience of "carotid loop" method. ....	47.
Personal experience of "ear artery" method. ..	59.
Personal experience of "abdominal band" method. ....	61.
Review of the "pressor body" hypothesis of hypertension. ....	66.

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### VOLUME 11.

Experimental methods of producing vascular hypertension. ....	107.
Experimental oxalate nephritis. ....	149.
Personal experience of oxalate nephritis. ....	159.
The hypertension of oxalate nephritis. ....	166.
The blood pressure in bilaterally nephrectomised rabbits both with and without oxalate injections. ....	175.
Renal denervation. ....	181.
Effect of renal denervation on the hypertension of oxalate nephritis. ....	194.
Experimental glomerulo-nephritis and hypertension. ....	198.
Personal experience of serum nephritis. ....	206.
Research into the hypertension of serum nephritis. ....	219.
Summary and conclusion. ....	229.



THE EXPERIMENTAL PATHOLOGY  
OF RENAL HYPERTENSION.

"As is our pathology, so is our practice".  
Osler.

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With the publication by Richard Bright in 1836 of his "Cases and Observations Illustrative of Renal Disease, accompanied with the Secretion of Albuminous Urine" there was initiated a ceaseless search for the exact relationship between renal and cardiovascular disease. In this famous paper Bright recorded the results of his clinical study of 100 patients, correlated with the post-mortem observations. His study revealed a coincidence of cardiac and renal disease so striking as to convince him that the cardio-vascular disease must be a more or less direct consequence of the renal disease. It must be remembered that cardiac hypertrophy was the only index of hypertension which was available to the physicians of that time, as it was not until some 60 years later that the use of the sphygmomanometer became at all general. It would not be out of place to quote some of Bright's comments upon the relationship/

relationship of cardiac and renal disease.

"The deviations from health in the heart are well worthy of observation: they have been so frequent as to shew a most important and intimate connection with the disease of which we are treating; while at the same time there have been twenty-seven cases in which no disease could be detected; and six others, which, from not having been noted, lead to the belief that no important deviation from the normal state existed. The obvious structural changes in the heart have consisted chiefly of hypertrophy, with or without valvular disease; and, what is most striking, out of fifty-two cases of hypertrophy, no valvular disease whatsoever could be detected in thirty-four. .... This naturally leads us to look for some less local cause for the unusual efforts to which the heart has been impelled; and the two most ready solutions appear to be, either that the altered quality of the blood affords irregular and unwonted stimulus to the organ immediately; or, that it so affects the/  
the/



the minute and capillary circulation, as to render greater action necessary to force the blood through the distant subdivisions of the vascular system."

Although 100 years have elapsed since the publication of this paper, the exact relationship between renal disease and vascular hypertension is still unknown. This has not been due to any lack of investigation but rather to the inherent complexity of the problem. Not the least of the difficulties confronting the experimental investigator in this subject has been the great difficulty of making repeated reliable observations of blood pressure in experimental animals. Therefore an essential preliminary to any investigation of this subject is a consideration of the methods available for the measurement of blood pressure in laboratory animals.

#### Estimation of Blood Pressure in Laboratory Animals.

Direct Methods: The essence of this method is the insertion into an artery of a cannula and the direct measurement of the intra-arterial pressure. It was first employed by Stephen Hales (1773), who introduced/

introduced a piece of brass piping into the left femoral artery of a horse; attached to this pipe was a length of glass tubing 9 feet high and  $1/6$  inch in diameter. The blood rose in the tube 8 feet and 3 inches above the level of the left ventricle. The disadvantages of this method were, of course, the awkwardness of the long tube and the rapid coagulation of the blood. Poiseuille (1828) solved both these difficulties. He introduced the U-shaped mercury manometer which reduced the space required for measurement to less than one thirteenth. He was able to retard coagulation by filling the tube leading from the artery with a saturated solution of sodium carbonate. Ludwig (1847) adopted the mercury manometer so that graphic records could be made. Since then direct methods have been elaborated by Hürthle (1890), Janeway (1904), and others. These methods are of great value in relatively short investigations, but there are many disadvantages - (1) anaesthesia and operative procedures are necessary, both of which are bound to cause some variation from the normal blood pressure in the animal; (2) the blood pressure can be observed for only/

only a few hours, therefore the method is useless in experiments extending over days, weeks, or months; (3) the artery has to be ligated following the taking of the tracing and can, therefore, not be used again for that purpose. Few other arteries in the same animal are available for a further experiment. For these reasons it is desirable to have a simple, accurate method of taking repeated measurements of blood pressure in laboratory animals. Several investigators have worked on the problem and various methods have been published.

Indirect Methods in Use on Animals: A review of the literature shows that the methods may be arranged in four groups:-

- (1) Readings on peripheral vessels with a tonometer.
- (2) Readings on a central artery of a rabbit's ear.
- (3) Readings on the carotids.
- (4) Readings on the aorta and femoral arteries.

Tonometric Methods.

Gärtner (1899) made a practical use of Marey's original observation of the return of colour to the skin with the release of pressure on the artery of the tails of dogs. Trendelenburg (1913) published

supply. This constitutes the first record of indirect measurement of blood pressure on an animal. This method is also applicable to man.

Apparatus: The apparatus consists of (1) a pneumatic ring made up of a metal ring 1 cm. high and 2.5 cm. in diameter, the inside of which is clothed with a rubber bag which can be filled with air; (2) a rubber bulb; and (3) a T tube uniting (1) and (2) with a mercury manometer.

The method is as follows:- A finger is first made anaemic by pushing it into a compression apparatus such as immersion in mercury. The pneumatic ring is placed over the second phalanx and the pressure in it is raised to a point higher than the blood pressure. The compression apparatus is removed from the terminal phalanx, leaving it pale and bloodless. The pressure in the ring is gradually lowered while the colour of the finger is carefully observed. At the moment when the blood enters the pale part of the finger colouring it purplish-red, the pressure on the mercury manometer is identical with the blood pressure. Gärtner (1899) used the same principle to read blood pressures on the tails of dogs. Trendelenburgh (1913) published a/



a description of the application of the tonometric method on the fore-leg of cats. Only cats with clear skin were suitable for use.

Backmann (1916) applied a modification of this method to rabbits. His apparatus was the same as Gärtner used. According to this method, as the rosy skin of rabbits is normally hidden by long hairs, one of the posterior extremities is shaved. The distal part of the limb is rendered anaemic by the application of a bandage. The pneumatic ring is applied above the bandage. The pressure is raised higher than the arterial pressure and is gradually lowered 5 mm. of mercury at a time. When the skin suddenly becomes red the reading is taken. This indicates the normal blood pressure in the femoral artery. Backmann's figures for the normal averaged from 65 to 85 mm. of mercury. As many as thirty repeated observations in fifty minutes showed that blood pressure does not maintain an exact level. Comparison with the direct method gave almost parallel results when a cuff 35 by 18 mm. was used. The variations with different widths of cuffs were as much as 50% of the pressure.

More recently, van Eweyck and Schmidtman (1922)/

(1922) published a report of a tonometric method for use on rabbits. They stated that an observation of the capillaries of the skin is not possible even after the area is shaved. However, they found that the smallest vessels running in the fascia could be made visible and the return of blood could be observed if a small opening (the size of a pea) were cut in the skin. They had difficulty in fitting the cuff on the conical thigh of the hind leg and could not obtain satisfactory results. For that reason they used the foreleg. Their apparatus consisted of a rabbit board, a rubber bandage to produce the anaemia, the tonometric cuff to be fitted round the upper leg, and a manometer and rubber bulb with tubing and connections. The rabbit was fastened on the board in the dorsal position. One of the forelegs was rendered anaemic by means of the rubber bandage. The cuff connected with the bulb and manometer was placed around the upper leg and the pressure then raised to about 160 mm. Hg. The rubber bandage was unwound. A small incision through the skin was made with scissors at some point below the cuff. The pressure in the cuff was gradually released. At a definite point/

point the blood returned to the small arteries of the fascia. A short time later the borders of the wound began to bleed. After taking the pressure the wound was closed with a suture and swabbed with iodine. Suppuration did not occur.

After taking many readings van Eweyck and Schmidtman concluded that the commencement of bleeding from the wall of the wound was a more accurate criterion of the systolic pressure than the reappearance of blood in the vessels. They also found that accurate results could be obtained only if the pressure was allowed to fall slowly. It should take one minute to fall 10 mm. of mercury. The readings were not taken until the excitement produced by tying the animal had subsided. Consecutive readings gave results within 2 mm. of one another. The average pressure in the foreleg of normal rabbits was from 90 to 100 mm. Comparisons with the direct method were made in four animals and the direct readings corresponded with the minimal pressure.

Schönheimer (1924) using the procedure of van Eweyck and Schmidtman (1922) found the normal range to be from 80 to 112. Von Deicke found the method fairly/

fairly satisfactory but felt that the necessary manipulation must influence the blood pressure. Thölldte stated that he had great difficulty in accustoming rabbits to the procedure.

Kunstmann (1928) used a tonometric method in mice, rats, and rabbits. The animal was extended on board or held in a special wire frame which did not enclose the rear limbs. A small rubber bag was wrapped round the thigh and by means of a pressure bulb connected to a manometer, the arterial blood flow in the limb was occluded. The pressure was released and the reading noted at the point where the vessels on the dorsum of the foot changed from white to red; a hand lens was used to observe the skin and in the case of hairy animals depilating was necessary. A modified form of the apparatus was used to observe the arterial pressure in a rabbit's ear.

Griffiths (1934) used a method in rats very similar to that just described. He depilated the dorsum of the foot and placed a drop of immersion oil on the skin and observed the vessels with the aid of the low power objective of the microscope. He stated that two types of vessels are to be seen in the skin, - (1) very red and distinct vessels with relatively/



relatively wide channels rarely showing any segmentation in their homogenous stream and flowing either very slowly or not at all; (2) relatively thin and faint vessels in which the flow is very fast and the stream granular in appearance. He concluded that readings should only be made from the latter type of vessel. The pressure in the cuff is slowly raised until the flow ceases, after which it is lowered 3 - 5 mm. at a time and the point noted at which definite flow begins in the thin vessels. He states that the end point is usually sharp. Observations made on a hundred rats varied between 140 and 60 mm.Hg., 90 percent of them lying between 80 and 100 mm. of the systolic

The chief advantages of the tonometric methods of determining blood pressure are:- (1) their simplicity; (2) the fact that they rely on one's most accurate sense, that of sight; (3) as many readings as desirable may be taken; and (4) anaesthesia or operation is not necessary.

The disadvantages are numerous. (1) It is impossible to obtain both systolic and diastolic pressures. (2) In conditions of low pressure the colour does not return at all. In normal animals the/

the flush may appear so gradually that it is impossible to get a reading. The time at which the flush appears depends partially on the rate at which the pressure is lowered. With too rapid lowering there is an under-estimation. Often, with a very slow release, a second deeper flush appears a few millimeters of mercury lower. Martin (1903) found differences up to 20 mm. between the two flushes. This has been confirmed by other workers. (3) The vessels observed are small and are subject to vasomotor influence. At best the results only indicate the pressure in the small peripheral arteries. Variations of pressure in these may be purely local and may not be any indication of the systemic condition. With successive determinations the readings become higher and higher, more so than with the Riva-Rocci apparatus. This may be due to continued compression initiating a vasomotor reflex. (4) Shapiro and Seecof (1924) criticised the van Eweyck - Schmidtman (1922) method because the struggling entailed in tying the rabbit to a board may increase its pressure. However, Schmidtman stated that readings must not be taken until the excitement has disappeared. Shapiro and Seecof also suggested/

suggested that the incision in the skin may influence the blood pressure in that limb by changing the blood flow to the part, and finally they point out how difficult it is for one person to watch the incision and note the blood pressure at the same time. The necessity for an assistant is certainly a disadvantage. (5) Perhaps the most obvious objection to the tonometric method is the lack of correspondence of results. Backmann's readings were taken on the posterior extremity of rabbits, while Schmidtman's were taken on the foreleg. The vessels in these two limbs should have almost the same pressure, yet Backmann's figures were from 65 to 95 mm. Hg. while Schmidtman's were from 90 to 100 mm.

The fact that such reliable workers can obtain such different values with practically the same method is to the discredit of the tonometric method.

#### Central Artery of Ear of Rabbit.

Von Recklinghausen (1906) described a non-surgical method for the measurement of blood pressure in the ear of a rabbit.

Apparatus: The apparatus consists of a flat inflatable rubber ring, shaped like a washer, and connected with a rubber bulb and a manometer, a glass/

glass plate, a spherical glass flask, such as is used in chemical laboratories, and a watchmaker's lens.

The inner side of the ear lies against the flask. The hairy outside of the ear is shaved over the proximal portion of the central artery. On this the rubber bag with the hole in the centre is placed so that the artery can be observed through the hole. A glass plate is applied over the rubber ring. The observation is carried out by means of the light through the lens. The course of the light is as follows:- the flask, inner side of ear, ear substance with vessels, outside of ear, hole through the rubber ring, glass plate, lens, eye of observer. The flask, ear and glass plate are held with the left hand while the pressure in the bag is controlled with the right hand. An assistant reads the manometer. By varying the pressure in the rubber ring von Recklinghausen was able to see the vessel fill and empty. With a strong lens he observed the course of single corpuscles and he could distinguish systolic pressures. He advised using a strong lens and only slightly pigmented animals. He noted the widening of the vessel in a warm room. The method does not seem/



seem to have been much used, chiefly because it was difficult to control the pressure applied. However, Anderson (1923) and Tohoru Kuraya (1923) independently worked out and published modifications of von Recklinghausen's procedure.

Anderson's method is as follows:-

Apparatus: The apparatus consists of a specially designed U-shaped pressure piece. One end is covered with a thin rubber membrane and connects with a mercury manometer and bulb. The other end contains a bright electric light such as is used in an ophthalmoscope. A lens is attached to the back of the pressure piece to magnify the vessel. Pressure is applied by means of a rubber bulb attached to a manometer.

The rabbit's ear is slipped into the apparatus so that the proximal portion of the central artery lies between the rubber membrane and the source of light. When the light is on, pulsations may be observed in the vessel. As the pressure is raised the pulsations cease. The level of mercury at which this occurs is taken as the systolic pressure. Anderson stated that the readings must always be taken at the same point in the vessel since the pressure decreases peripherally/

peripherally. He found that excitement, such as lifting the rabbit by the ears, gave an average rise of 13.3 mm. Hg. in pressure. Vasomotor changes altered the pressure; so he kept the ear covered with a small muff. External heat raised the pressure 20 mm. or more. He found that young rabbits tend to have a lower pressure than adults. His results were consistent. The average systolic pressure in the central artery of a rabbit's ear by this method varied from 76 to 87, with extremes of from 70 to 90. These figures were confirmed by Shapiro and Seecof (1924) in their experiments on the influence of experimental atherosclerosis on the systolic pressure in rabbits. (1927) described a similar type of

In the same year Tohuru Kuraya (1924) published a report of a similar method.

Apparatus: The apparatus consists of a glass plate, a rubber drum, and a manometer.

Procedure: The ear of the rabbit is shaved and the reading is taken in a warm room to dilate the vessel. An anaesthetic is given to keep the animal motionless. The inner side of the ear is placed next the glass plate and the outer surface is in contact with the rubber drum which is connected with a manometer. The blood stream is stopped by raising the pressure in the/

the drum. As the pressure is released the blood flow is resumed. This level of mercury is equal to the maximum or systolic pressure. An electric light may be attached to assist in observing the vessel. The blood pressure in the central artery of the ear in normal rabbits varies from 35 to 50 mm. of mercury. It is always from 56 to 63 mm. lower than the carotid pressure.

It is to be noted that Anderson's (1922) figures for the normal were from 75 to 90, while Kuraya's (1924) were from 35 to 50. The lower figures may have been obtained from a distal part of the artery.

Squier (1927) described a similar type of apparatus in which a brass drum, covered at one end with sheet rubber, was employed. Inside the drum was a small electric lamp. The shaved ear was placed with the inner side next the drum and a piece of plate glass was placed on the outer side and held in position by two screwed rods attached to the base board on which was mounted the drum. Readings were taken by raising the pressure in the drum by means of an inflator attached to a mercury manometer. The systolic pressure was taken as the point at which the blood/

blood ceased to flow in the central artery. This point could be observed accurately with a lens. His readings ranged from 60 to 70 mm. Hg.

Behrens (1926) published a report of a similar method which he had worked out and used in 1923 independently of Anderson (1922) and of Kuraya (1926).

Apparatus: The apparatus consists of a glass plate, a rubber membrane stretched over an artery and a Mareyscher glass case which is connected with a rubber bulb and a manometer. The ear is placed in the small apparatus between the glass plate and the rubber membrane. The pressure is raised and the ear is pressed against the glass plate. The central artery and vein are clearly visible. As the pressure increases the vein disappears and only the pulsations in the artery can be seen. The reading is taken at the point at which the pulse disappears. His values for the normal - about 40 mm. Hg. - agree with those of Kuraya (1924), and so were probably taken on the same portion of the artery. Behrens (1926) pointed out that the chief disadvantage is due to the small size of the artery of the ear. External stimuli could cause such marked vascular constriction that a reading of blood pressure would be impossible.

Watson/ long and 10 mm. outside diameter, the

Watson (1927) published another modification of von Recklinghausen's method. The principle is the compression of the central artery of the rabbit's ear by a rubber membrane attached to a pressure chamber on an illuminated stage. The pressure chamber is connected with a mercury manometer and bulb. The artery is observed through a lens focussed on the stage. Watson's figures for the normal correspond with those of Anderson (1922) and of Shapiro and Seecof (1924).

Neumann (1930) published a further modification of the von Recklinghausen method, and this modification was improved by Schmidt-Weyland (1931). This apparatus was certainly excellent for observations of the rabbit's ear but it suffered from the special disadvantage that two operators were required.

One of the most recent publications on the subject of blood pressure registration in rabbits is by Grant and Rothschild (1934). It is also based on von Recklinghausen's method and it employs the central artery of the ear.

The device consists of an airtight pressure capsule and a support by which it is applied to the ear. The capsule A. is a cylinder of aluminium tubing 10 mm. long and 19 mm. outside diameter, the thickness/



thickness of the wall being 1.5 mm. The upper end of the cylinder is recessed internally and into this is cemented a glass circle. The lower end of the cylinder is grooved externally and is covered by Cargile membrane, which is tied on this groove. The membrane is lax. A brass tube B, 3 mm. outside diameter and bore 1.5mm, length 15 mm., is screwed into the middle of one side of the capsule. On the outside of the tube a screw thread is cut. The support is a U-shaped strip of aluminium 1.5 mm. thick and 20 mm. wide. The gap between the limbs of the U is 2 mm. The lower limb C is 55 mm. long and into its free end (beneath the capsule) is inserted a glass circle 16 mm. diameter. The upper surface of the glass is flush with the aluminium. The upper limb of the support E and F is 40 mm. long; 10 mm. from its free end two cuts each 5 mm. long are made at each side. The last 10 mm. of aluminium F are turned vertically upwards to these cuts and then bent into an arc of a circle to fit the outer side of the capsule. The capsule is held firmly to the aluminium arc by a nut and washer on the brass tube. The capsule tube is connected by rubber tubing to a bottle of 2 litres capacity and through this to a mercury/

mercury manometer and rubber pressure bulb.

r.d/r  
To use the capsule, either the ear is previously epilated or the hair is cut short over the central artery towards the base of the ear. The membrane of the capsule is moistened with 70% glycerine. The thin postero-lateral margin of the ear is slipped between the limbs of the support until the central artery lies beneath the centre of the capsule. When the limbs of the support are adjusted to grip the ear gently, the light capsule remains in position without being held by hand unless the animal is restless. The ears may be kept erect by fastening them together with adhesive plaster applied to their anterior margins. The artery is viewed through the capsule and the pressure is gradually raised. The lax membrane, filling out, presses on the skin over the artery. As the intra-capsular pressure is raised the arterial blood column becomes reduced in diameter, pulsates freely and is then interrupted first in diastole and finally, after a further pressure rise of 1 or 2 mm., in systole also. The pneumatic pressure required just to break the column of blood in systole is taken as the systolic pressure in the artery/

artery. This end-point is readily obtained and if respiration is not too rapid, even the small rise and fall of blood pressure with expiration and inspiration can be observed.

The authors point out that the ear artery shows great variation in calibre. For instance, a diameter of 10 mm. may be reduced to only 0.1 mm. if the ear be cooled or the animal be startled. They compared the ear pressure in the anaesthetised animal with that measured by a mercury manometer through a cannula in the carotid or femoral artery; the results showed that, provided the ear artery be not greatly constricted, the systolic ear pressure lies 10 to 15 mm. below the mean carotid pressure and is the same as mean femoral pressure. The required degree of arterial relaxation is attained by keeping the animal sufficiently warm. It may also be attained by denervating the ear. However, this must be freshly done, for Grant and Bland (1933) have shown that following denervation the vessels of the rabbit's ear undergo a complex series of changes. In the unanaesthetised animal, warm and resting quietly, the blood pressure remains almost steady for long periods and lies between 70 and 90 mm. Hg.; if excited/

excited or restless, the pressure may rise to 100 mm. Hg. or more. When care is taken to make estimates under standard conditions of rest and warmth they consider that the pressure remains remarkably constant from day to day. Considerable constriction of the ear artery materially lowers the pressure necessary for occlusion. Thus in a rabbit with one ear normal and the other ear freshly denervated the pressures were measured simultaneously with a capsule on each ear. The pressures in the two ears were equal when the animal was warm, but when the animal was cooled the vessels of the normal ear constricted greatly and the pressure in this ear fell 10 mm. below its previous level; the vessels of the denervated ear remained unchanged and the pressure here rose 10 mm., probably as the result of vasoconstriction elsewhere. On rewarming the animal the pressures once again became equal.

These workers further noted that the state of the smaller vessels of the ear has a definite though slight effect on the systolic pressure in the main artery, constriction causing a rise, and relaxation a fall. For example, in the cooling experiment already described the fall of pressure in the normal ear/

ear was preceded by a rise which occurred owing to constriction of the minute vessels and before the main artery became obviously reduced in calibre. So also a subcutaneous dose of adrenalin acting slowly on the vessels gives first a rise of pressure when the minute vessels constrict, followed by a fall when the dose is sufficient to constrict the main artery.

Further, in a warm and resting animal with one ear freshly denervated the pressure in the two central arteries are equal; after a few days, when tone has been regained in the denervated ear and it is paler than the normal ear, the pressure in the denervated ear is usually a little higher (5 mm.Hg.) than that in the normal ear. Similarly, occlusion of the central artery of the normal ear distal to the capsule raises the systolic pressure slightly. Again, the slight fall of pressure that is found when estimations are made in rapid succession is to be attributed to relaxation of the distal vessels.

When the animal is warm or the ear is fully denervated, the main ear arteries are fully relaxed but the minute vessels though dilated are not maximally so. They can be induced to dilate further by/



by several methods; for example, occluding the central artery for even a few seconds produces temporary flushing because of reactive hyperaemia. This flushing of the ear is seen when the pressure is released in the capsule after the artery has been occluded, and if pressures are taken during this phase a drop of 5 mm. will be found. However, the vascular reaction subsides in one minute.

The advantages of the method of von Recklinghausen or any of its modifications are, - (1) its stability and the ease with which numerous readings may be taken; (2) its dependence on the observer's visual sense. The disadvantages are, - (1) the figures for the normal rabbit quoted by Anderson (1922) and by Kuraya (1924) do not agree, although Anderson's results have been confirmed by several workers (Shapiro and Seecof, 1924); (2) there is the same objection to readings on the central artery as there is to those taken with a tonometer. Changes in pressure in such peripheral vessels may be local only (due to vasomotor influence) and not indicative of general blood pressure. In spite of the use of ear muffs or a room of constant temperature, it is difficult to control/

control the calibre of the vessel.

Carotid Loop.

Van Leersum (1911) reported an ingenious method for taking the blood pressure of rabbits. He selects young animals and performs an aseptic operation under ether anaesthesia, thereby isolating and placing the carotid artery within a skin tube. Two longitudinal parallel incisions from 7 - 10 cm. long and about 3 cm. apart are made in the neck. The skin between the incision is freed from the underlying muscle. The carotid artery is isolated from its surrounding structures for several centimetres. The edges of the strip of skin are sutured together enclosing the artery in the tube thus formed. The outer edges of the two incisions are then sewed together. The incisions should heal by first intention and the stitches may be removed within a week.

Method as advocated by van Leersum: A rubber cuff 1 cm. wide and from 3-4 cm. long is connected by rubber tubing to an air pump and a manometer.

Procedure: Assistant holds the rabbit, keeping the feet in one hand and the head in the other. The narrow/ pressure are around 130 mm. Hg. He

narrow rubber cuff is then wrapped around the carotid tube. Air is forced into the cuff until the artery is occluded and then the pressure is gradually released. A finger on the carotid tube distal to the cuff palpates the first pulse wave that comes through. A second assistant, at a signal, notes the level of mercury and records that as the systolic pressure. In this way from three to five readings a minute can be taken.

Van Leersum advised that readings be taken as soon after the operation as possible. The manipulation promotes the absorption of inflammatory products. If readings are not taken for six weeks the tube becomes hard and stiff. The procedure should be carried out in a quiet room and the animal should be handled gently. The first few compressions of the tube exert a massaging effect and soften the skin so that less pressure is necessary to close the vessel. In excitable animals these first readings are always higher than those which follow. It is advisable to disregard all values obtained until the animal is quiet and the tube softened. Van Leersum's figures for the normal carotid pressure are around 130 mm. Hg. He compared/

compared his method with readings obtained with a cannula in the carotid and also in the femoral artery. The direct values for the femoral artery were 10.7 lower, and for the carotid 10.4 lower. The difference can be accounted for by the type of mercury manometer used, which records only mean pressure. The advantage of the van Leersum technique is that it is applied to an artery of large size. The values obtained should therefore indicate the general blood pressure of the animal.

Van Leersum himself points out the main disadvantage of his method. The thickness of the wall around the artery alters the amount of pressure required to close off the column of blood. For that reason he was surprised to find that there were no great discrepancies between the direct and indirect methods of taking pressure. He considers that if the carotid tube could be constructed without altering the compressibility of the arterial wall by inflammatory reactions the procedure would be more reliable. The fact that an operation must be performed is in itself a disadvantage. Even if the wound heals the artery may become thrombosed. After healing, the artery is/

is in an exposed position and is always subject to trauma. This may result in haemorrhage and the animal's usefulness for determinations of blood pressure is past. The necessity for two assistants hampers the procedure and leads to inaccuracy.

Cohn and Levy (1920) reported a modification of the van Leersum method. They used the same procedure, but they added to the apparatus. They have a graphic method of recording the pressure. The system between the cuff and the manometer is filled with water. The record is made on smoked paper by a writing point supported on a float on the mercury column.

Dominguez (1924) published another modification of the van Leersum technique. The same operation is performed to obtain a carotid tube. Apparatus: The apparatus consists of a cuff 1.5 cm. wide made of soft surgical drainage tubing and a Riva-Rocci manometer.

Procedure: The rabbit is placed in a box with its head through an opening in front. The animal cannot slip in or out, so an assistant is not necessary. The cuff is placed round the carotid loop and fastened with a bulldog clamp. The readings are taken/



taken as in the original van Leersum method.

Dominguez (1924) reported blood pressure observations on 63 animals, and from 50 to 6,000 observations on each. His routine for each animal consists of ten readings, the taking of its pulse and the record of the temperature of the room. This requires about five minutes. His results are carefully controlled with regard to the effect of haemorrhage and the effect of injections of morphine. His figures for normal carotid pressure in rabbits vary from 90 to 150. He compared his method with the pressure obtained from a cannula in the femoral artery. His readings average 18 mm. higher. When he used a maximum and minimum manometer in the other carotid artery, his readings were only from 10 to 12 mm. higher. He concluded that the method gives the real value of blood pressure with sufficient accuracy to render comparable the results obtained from day to day.

The advantage of the Dominguez modification lies in the fact that only one person is required to make the observations. The disadvantages depend on the complications of the operation which he himself reports. The skin flap often becomes necrotic/

necrotic. The scar tissue contracts and obliterates the artery. When infection occurs in the loop the artery often becomes thrombosed. There was a 20.8 per cent mortality rate in a series of 96 operations; 21 more animals could not be used because of necrosis or infection. Thus only 57 per cent of the operations were successful. This is a high mortality rate at the beginning of the experiments without considering the accidents in which the carotid loop may later become involved.

Behrens (1926) reported still another modification of the van Leersum technique. The carotid loop is obtained by the usual operation.

Apparatus: The apparatus consists of a glass tube from 8-10 mm. in diameter, with a hoop-shaped attachment on one end for the reception of the carotid. At one end of the tube is a thin rubber membrane which on inflation lies against the hoop-shaped process and pinches off the carotid. The apparatus has exactly the principle employed by Anderson (1922-23) which was used on the rabbit's ear. The rest of the apparatus consists of a rubber bulb and mercury manometer. The apparatus is applied as centrally as possible and at right angles to the loop/

loop so that the artery is not pulled from its natural position. The pressure is raised until the pulse in the carotid disappears. This is estimated by palpation. An assistant reads the manometer and records the pressure. Behrens (1926) gave the average carotid pressure in normal rabbits as 90 mm. of mercury. In some cases it was as high as 120 mm. Comparison with the cannula method gave a maximum error of 5%. This method requires an assistant and does not have any particular advantage over the procedure of Dominguez or of van Leersum.

Koch and Mies (1928) described a method of utilising the carotid artery for repeated observations of pressure over a period of several hours. They used rabbits. The procedure consisted essentially in the preliminary operative trans-plantation of the carotid artery above the muscles of the neck in such a way that a metal chamber containing a glass window could be clamped over the artery. One side of the chamber was covered by a rubber membrane and the artery was placed so as to lie between the membrane and the glass window. Air pressure was applied against the membrane until the column of blood in the artery was just obliterated, and/

and the pressure was then read from a mercury manometer. After applying the chamber it is advisable to wait 15 minutes before taking readings.

Petroff (1929) used the carotid loop method in dogs and he described a modification whereby the return of pulsations in the loop could be observed by an auscultatory method. Provided he was careful to protect the dog from exciting stimuli he found the method was satisfactory, the normal pressures ranging from 118 to 130 mm.Hg.

Schlesinger (1930) described a two stage operation for the construction of a carotid loop. At the first operation the carotid artery was dissected free and enclosed in a fold of skin raised from the front of the neck. Eight to ten days later the fold of skin was separated so as to form a tube and the skin edges united behind the loop. He claimed that with this method necrosis of the skin of the loop never occurred. He compared the pressures observed by the method of Koch and Mies (1928) with those observed by the carotid loop method and he found that whereas the former gave results which agreed exactly with intra-arterial observations, the results with the latter were approximately/

approximately 20 mm. higher. However, although different, the carotid loop readings exactly paralleled the absolute values.

Kottlors and Rothschild (1932) in describing a cuff method advance some vague criticisms of the carotid loop method based on supposed interference with the carotid sinus. They further state that in constructing carotid loops they only had 23 per cent of successes, owing to frequent thrombosis, necrosis, and sepsis.

The carotid loop method has been used with success by several recent investigators including Hartmann, Bolliger, and Doub (1926), Kremer, Wright, and Scarff (1933), Hanzal, Goldblatt, Lynch, and Somerville (1934), and Page (1935).

#### Cuff Methods.

Erlanger (1904) succeeded in reading the blood pressure in the femoral artery of dogs by an indirect method.

Apparatus: Erlanger's sphygmomanometer, which is the most accurate instrument of its kind available, was used. The width of the cuff is from 6.3 to 9 c.m. It was found that the application of the cuff to the conical shape of a dog's thigh was difficult/



difficult. The cuff was dragged down on the leg as the pressure was raised, thus losing much of the force. To eliminate this difficulty the limb was made cylindrical by excising the muscles on the dorsal and ventral aspects of the thighs of large dogs. The excess of skin was removed and the wound allowed to heal. Cuffs of different widths were applied to the thigh. The systolic pressure was estimated by palpating the pulse in the femoral artery or by Erlanger's graphic method. The extensive operation involved in this procedure prevents its being a practical method. The pressure is slowly

Fahr (1912) reported a new technique for taking readings of blood pressure in rabbits. at the artery

Apparatus: The apparatus consists of a cuff of the Riva-Rocci type connected with a rubber bulb and a mercury manometer. The cuff is wrapped round the abdomen of the animal. The pressure in it is raised until the abdominal aorta is occluded. The pressure is gradually reduced and by palpation of the femoral artery the first pulse wave is noted. This level of mercury is equal to the aortic systolic pressure. In their criticism of this method Shapiro and Seecof (1924) stated that constriction of the rabbit's abdomen/

abdomen is unphysiological. A more important and practical objection is the difficulty of palpating the femoral artery in so small an animal.

Janeway (1904) reported a simple means of obtaining measurements of blood pressure on dogs. Apparatus: The apparatus consists of a modified Riva-Rocci cuff, one 7.5 by 15 cm. being used for large dogs, and one 5 by 11 cm. for small dogs, and a pressure bottle connected with the cuff and manometer through valves operated by foot pedals. This leaves both hands free. The cuff is wrapped round the lower foreleg. The pressure is slowly increased to above the systolic pressure. The obliteration of the pulse is palpated at the artery at the bend of the ankle or on the plantar surface of the paw. Janeway stated that it is impossible to palpate the return of the pulse after obliteration. Even obliteration of the pulse can be detected only in those dogs with a large superficial artery. When the animal is quiet, the pressure can be read with a maximum error of 15 mm. on the side of underestimation.

This appears to be a simple method which is applicable for the larger laboratory animals. An operation/

operation is not required, and the dog need not be tied. The only difficulty, then, is the palpation of the peripheral vessel. Janeway overcame this by careful selection of the animals.

Kolls (1920) published a report of an indirect method of determination of blood pressure on dogs.

Apparatus: After carefully studying the shape of a dog's thigh, Kolls devised a specially shaped cuff. For dogs weighing from 6 to 14 kg. the cuff is 14 cm. wide. For larger dogs, a longer and wider cuff must be used. He devised a special instrument to record the pulsations, because the ordinary sphygmograph was not delicate enough.

The part of the cuff which is inflexible and of sheet aluminium is placed over the external surface of the thigh. Pressure is applied over the internal surface, thus compressing the femoral artery. The cuff does not tend to be dragged distally, so pressure is not lost in this way. In comparison with the direct method a maximum and minimum valve cannula in the opposite femoral gave differences not exceeding 5 mm. This procedure is particularly valuable for use on dogs. It does not depend on palpation, which is often so unsatisfactory. It/

It would not be suitable for rabbits but is the method of choice in larger animals.

Götze used the ordinary cuff to obtain estimations of blood pressure on cattle. He applied the cuff to the tail of the animal, raised the pressure to above blood pressure, and then gradually lowered it. By palpation of the coccygeal artery for return of the pulse, he obtained the systolic pressure.

A report of another simple and accurate method for obtaining readings of blood pressure on dogs was published by Frederick Allen (1923).

Apparatus: An ordinary sphygmomanometer, with a cuff of the size used for infants, and a stethoscope with a phonendoscope bell piece, small and flat, about 2 cm. in diameter and 0.75 cm. thick, with a celluloid diaphragm, are used. There is a ring on each side to attach tapes.

The dog is kept in a standing position by a frame and canvas bands which pass under the abdomen. A strap holds the tail still. The lower part of the hind leg is shaved and the course of the femoral artery located. The stethoscope bell is attached by the tapes over the vessel. The cuff is wrapped around/

around the leg so that the lower border just covers the stethoscope bell. Readings are taken as in clinical practice. The cuff is inflated until the pulse is obliterated. As the pressure is lowered, the first sound indicates the systolic pressure and the fourth phase, when the sound suddenly fades, is the diastolic pressure. Observations are more easily obtained on animals that are accustomed to being handled. The sounds are usually clearer on large dogs. The method has been used successfully on sheep. Allen's figures for the normal average are 79 mm. Hg. diastolic and 139 mm. Hg. systolic, with a pulse pressure of 60 mm. Control observations were made with a cannula and an ordinary manometer, and there was a satisfactory parallelism between the readings. Allen's method, like that of Kolls, is dependable for dogs. In smaller laboratory animals, however, the sounds of the femoral vessels cannot be heard with a stethoscope.

Biasotti (1927) used a similar method with success.

McGregor (1928) reported a combination of the methods of Allen and Fahr.

Apparatus: This consists of an inflatable cuff, 12 cm. wide, connected with a rubber bulb and a mercury/



mercury manometer, such as is used in clinical work; a stethoscope with a flat chest piece 3 cm. in diameter and 0.75 cm. deep and provided with a diaphragm, and finally a rabbit board.

Procedure: The rabbit is tied on its back with the minimum of struggling or excitement. He states that it is possible to accomplish this by adhering to the following technique. Place the animal on its four feet on the board. Tie a cord about 18 inches long to each hind leg above the knee joint so that it will not slip off. Fasten the strap which is attached to the upper portion of the board around the rabbit's body behind the shoulders. The strap must fit snugly but not tightly enough to interfere in any way with the breathing. Holding firmly and steadily on the pelvic girdle, turn that portion of the animal on its back. The head and forelegs, although above the strap, will also rotate. Tie the cords attached to the rabbit's hind legs to the clips on the board. With a little practice a rabbit can be tied down in the supine position without causing it any discomfort. The animals soon accustom themselves to the procedure and/

and do not offer any resistance. The cuff is next wound round the animal's abdomen, so that its inferior border is just above the crest of the ilium. The chest piece of the stethoscope is then slipped under the lower border of the cuff in the mid line. In this way it lies over the termination and bifurcation of the abdominal aorta. If any difficulty is encountered in keeping the stethoscope in position, tapes should be attached to the chest-piece. Readings are taken exactly as in clinical practice. The cuff is inflated until the aorta is obliterated and the pressure is gradually released. The sounds are loud and clear; the first sound indicates systolic pressure and the sudden transition from the loud to the soft sound indicates the diastolic pressure.

McGregor considers it essential that the rabbit be tied down without any struggling. This is easily accomplished with a little practice. If the animal is excited it should be tied securely and left to lie quietly for at least five minutes. It is convenient to use two rabbit boards. One animal is tied down and the apparatus adjusted. While it lies there a second animal is tied on the other/

other board. Readings are then taken on the first rabbit by which time the second rabbit is ready for a similar procedure. It is important that the stethoscope be not more than covered by the lower border of the cuff or the readings will be higher.

Among the advantages of the method may be mentioned (1) an anaesthetic is not required; (2) the apparatus is simple and easily obtainable; (3) the criteria are the same as those in clinical practice; and (4) the sounds are loud and clear.

Among the disadvantages that the author points out are, - (1) the method cannot be used immediately after certain abdominal operations. It is necessary to wait two days or more to allow of time for healing; (2) the constriction of the abdomen may cause rupture of the surgical wound or may be so painful that the rabbit struggles. It has, however, been satisfactory two days after operation on the kidneys such as nephrectomy, experimental hydronephrosis, or ligature of the renal vessels.

McGregor's estimation of the normal blood pressure in the rabbit is 125 for the systolic pressure and 90 for the diastolic pressure. These figures are based on over a thousand estimations.

Comparison/

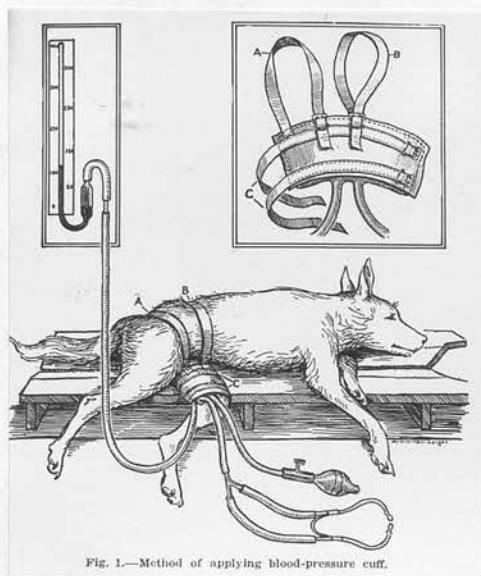


Fig. 1.—Method of applying blood-pressure cuff.

After Ferris and Hynes.

Comparison with the results obtained by means of simultaneous readings of pressure by means of a cannula in the common carotid artery indicates that the results run parallel. The indirect aortic pressure is about 10 mm. higher than the simultaneous reading of the direct carotid pressure, an ordinary end cannula being used. They are from 15 to 20 cm. higher than the intravascular aortic pressure between the taking of the readings.

Ferris and Hynes (1931) described quite a practicable cuff method suitable for dogs. They used large dogs (18 - 22 Kg.) and the rubber bag they used measured 13.5 by 22 cm. This bag was enclosed in a strip of cloth which was sewed to the central part of a heavy canvas strip which was designed to fit the tapering thigh of a dog. This strip measured 14 cm. wide, 40 cm. long on its upper edge, and 26 cm. on its lower. In order to overcome the tendency of the bag to slip down the conical thigh of the dog two webbing straps about one inch in width were fastened the whole length of the canvas strip with buckles so placed that the straps could be brought round the animal's leg and the cuff held in place. There were two additional straps attached at right angles to the horizontal straps/



straps. These passed round the dog's back and abdomen and completely prevented any tendency of the bag to slip. The stethoscope bell which was of the phonendoscopic type with a diaphragm 2.5 cm. in diameter, was placed just beneath the edge of the cuff over the artery which could be palpated posterior to the medial epicondyle of the femur. The manometer employed was a mercury instrument of Baumanometer manufacture. The left leg was used for estimations, the dog lying on that side without being strapped to the board. The hair on the medial side of the thigh was clipped so as to reduce adventitious sounds. The systolic pressure was recorded at the point at which the pulse sounds just became audible and was usually quite definite. The diastolic pressure was rather more difficult to determine since the sounds tended to fade gradually in some instances, instead of showing the sudden decrease in intensity which is usually taken as the diastolic pressure. Pressures were taken five times a week, and on each occasion an average of at least five readings were made on each animal. If considerable variation occurred more readings were made until four or five fairly constant values were obtained/

obtained. It was found that the pressure was elevated by such extraneous factors as the entrance into the room of a stranger, an unusual noise, or muscular contractions of the dog's leg. This indirect method was compared with direct cannula pressures on the opposite femoral artery, and the observations were found to be comparable within 5 or 10 mm. of mercury. It was further observed that the blood pressure of the dog tends to fall gradually during the first few weeks as he becomes accustomed to the procedure, until a relatively constant level is reached.

Maekawa (1930) and Rai (1930) have elaborated an apparatus for recording pressures in laboratory animals. An inflatable cuff is passed round a limb and it is connected to an oscillographic capsule of the Pachon type, optical recording being employed. In its arrangement the apparatus resembles that described by Golla and Antonovitch (1929) for a continuous recording of blood pressure in man. The apparatus suffers from the disadvantage that it requires very careful adjustment and is very easily disarranged. They found that the normal pressure in rabbits was 95-125/65-85.

The principal advantage of the cuff method is that it requires no preliminary operation. The main disadvantage/

disadvantage is that in animals smaller than dogs it is extremely difficult to follow the pulsations in the main artery compressed. In the dog it probably is the ideal method, a form similar to that used by Ferris and Hynes.

Personal Experience of the Carotid

Loop Method of Van Leersum.

This has been found to be the most satisfactory method of taking repeated blood pressure observations in the rabbit despite the technical difficulties and the apparently inevitable number of failures.

Method: Chinchilla rabbits provide the most satisfactory subjects as they have an abundance of loose skin on the anterior surface of the neck. Many of the earlier failures were probably due to the use of other breeds in which the scanty amount of subcutaneous tissue did not allow of sufficient collateral circulation to maintain the vitality of the loop.

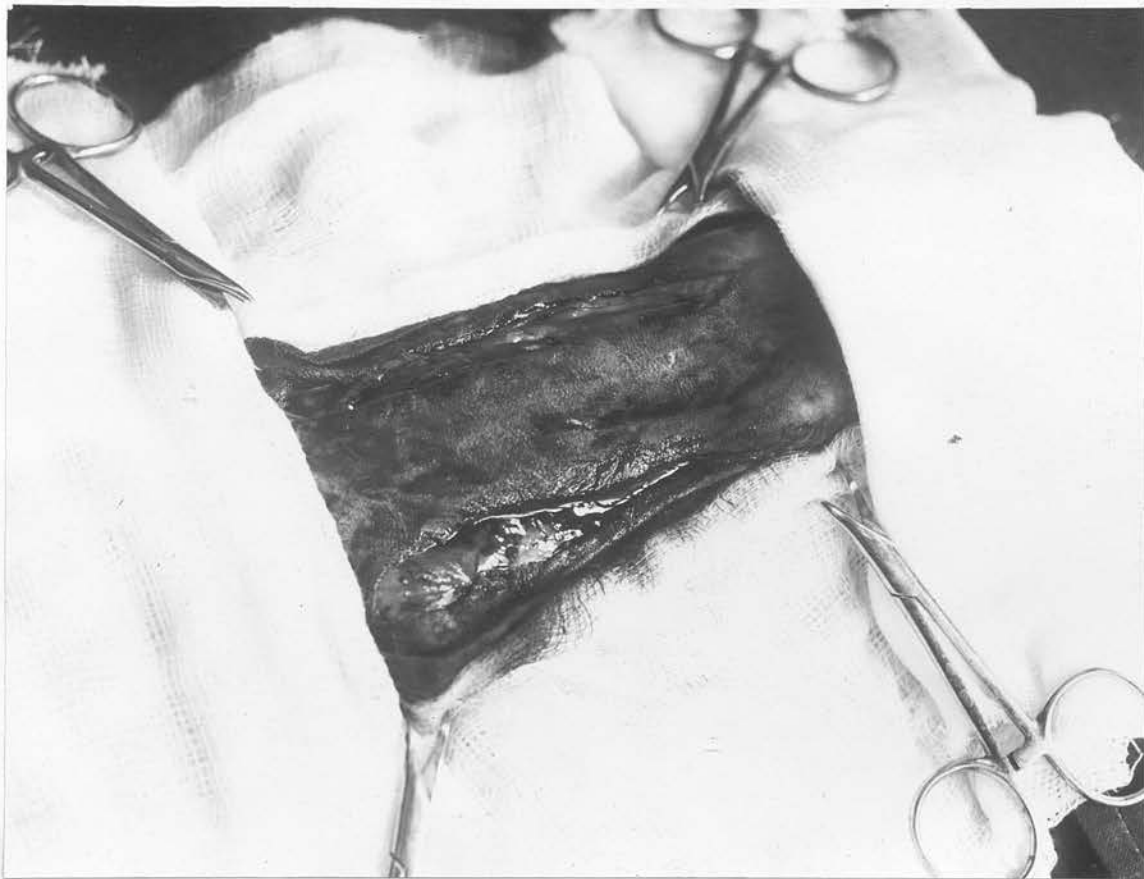
Care should be taken in the selection of animals to ensure that there are no scars or superficial skin lesions anywhere near the site of operation.

Anaesthesia is obtained by an intra-muscular or/

or intra-peritoneal injection of Nembutal in the proportion of 0.035 Gms. per Kgm. of body weight. A convenient way of obtaining such a solution is to dissolve 1 gm. of Nembutal in 28.7 cc. of sterile water, the dose then being 2 cc. per Kgm. of body weight. This dose produces sleep in about 25 minutes, although to obtain full surgical anaesthesia it is necessary to give a little ether, either with an open mask or pumped through a tube into the pharynx. On several occasions when Nembutal was not available, satisfactory operations were performed with ether anaesthesia alone, although there was a noticeably increased vascularity of the tissues, and excessive mucus secretion in the respiratory passages tended to impede respiration. Nembutal, however, is the most suitable anaesthetic as it enables the animal to be prepared during the period of sleep and it does not render the tissues vascular. Only ten deaths occurred during Nembutal anaesthesia in a series of 276 rabbits. Three deaths occurred from asphyxia with pure ether anaesthesia before the operation had begun; a tracheotomy was performed at once but the trachea was full of mucus. Autopsy showed pulmonary oedema.

A/





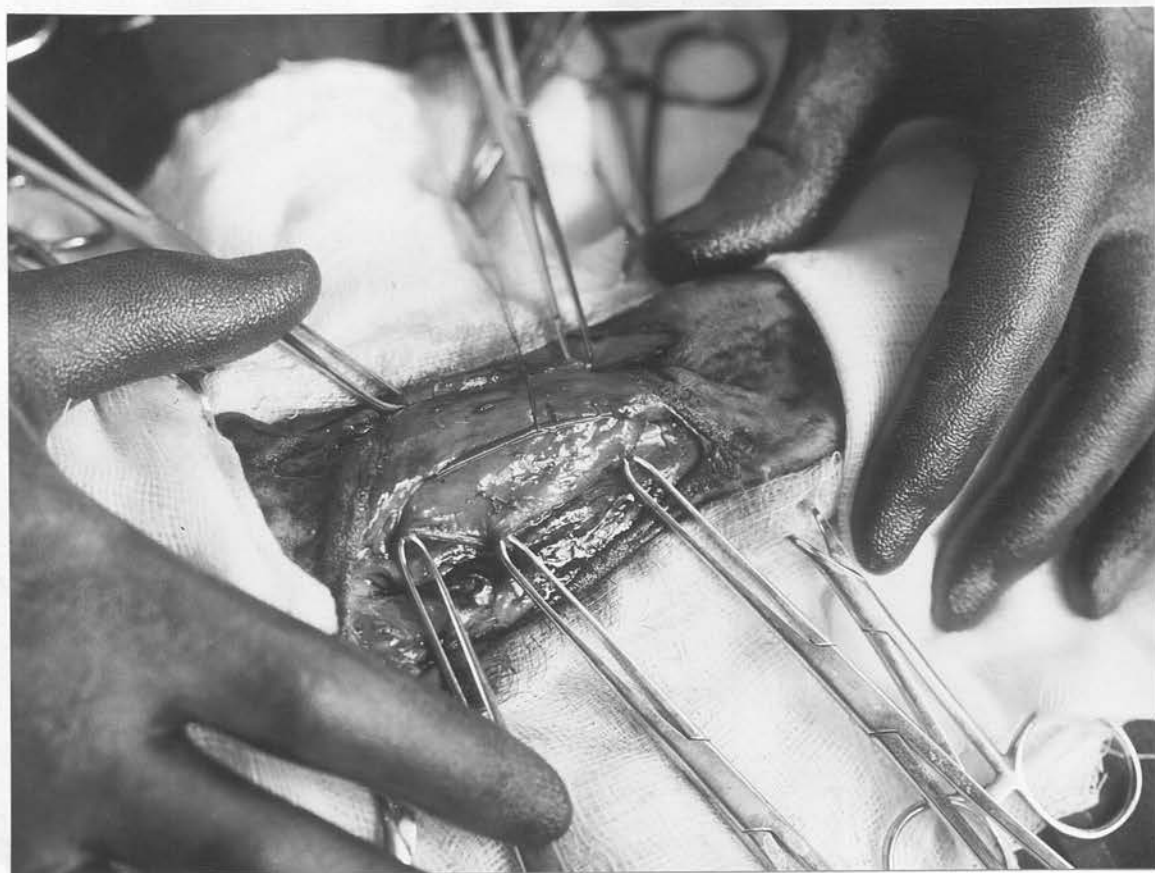
Preparation of Carotid Loop.

(1) Incisions Outlined.

A wide area of skin is shaved. We did several animals with a barium sulphide depilating paste, but we formed the definite impression that it tended to harden the skin and thus pave the way for subsequent atrophy and mummification of the loop.

In the actual operative procedure full surgical asepsis is employed. Instruments are boiled, knives, scissors, and needles are sterilised in pure anti-septol. Dressings, swabs, squares, gowns, caps, masks and rubber gloves are autoclaved. Silk sutures are sterilised in surgical spirit. The skin of the animal after shaving is washed with ether and then swabbed with iodine. The hands of the operator and of the assistant are washed for five minutes in warm water and fluid soap, with liberal use of a boiled scrubbing brush, and are finally rinsed in methylated spirit. The surrounding area is draped with sterile squares.

Operation: An incision is made on the left side of the neck from the angle of the mandible to about 1 cm. above the medial third of the clavicle; it is lightly curved with a lateral concavity. At this stage this incision is only carried down to the deep fascia. A similar incision is made on the right side with/



Preparation of Carotid Loop.

- (2) Strip of skin being wrapped round  
artery.

with this difference that it is placed about 1 cm. more laterally. There is thus mapped out on the anterior surface of the neck, more to the right side of the neck than to the left, a strip of skin with broad bases. The right incision is now carried through the deep fascia, care being taken to avoid the large jugular vein which should be retracted laterally. Search is then made for the division between the lateral border of the right sterno-hyoid muscle and the medial border of the sterno-mastoid muscle. Blunt dissection is used to separate these two muscles. There is now exposed the right carotid sheath with the vagus nerve lying antero-medial to the artery, the sympathetic trunk lying immediately behind and the depressor nerve lying anterior. The connective tissue of the sheath is carefully dissected away from the artery and a closed pair of artery forceps is passed under the vessel to use as a retractor. The vessel is dissected free as far up as the crossing of the nerve to the infra-hyoid muscles, and as far down as the crossing of the right subclavian vein. On several occasions this latter structure was wounded, necessitating its ligation, a procedure which tends to mitigate against the survival/



Preparation of Carotid Loop.

(3) Completed Loop.



survival of the loop. In dissecting the artery it is necessary to divide between ligatures the sup. thyroid artery. Having dissected the vessel, the incision on the left side is carried through the deep fascia and the strip of skin raised up from the anterior surface of the larynx and trachea. As much of the subcutaneous tissue as possible must be retained with the strip, otherwise it will tend to mortify. Any bleeding veins on the under surface of the strip must be carefully ligatured, otherwise a haematoma may result, - a mishap which has led to the failure of several operations through leading to the thrombosis of the carotid artery. We do not consider it necessary to suture the muscles behind the artery as is advocated by Kremer, Wright and Scarff (1933).

The strip of skin is now wrapped round the artery and the edges united with a series of interrupted silk sutures. Care is taken to handle the strip of skin as little as possible, as the application of tissue forceps has been observed to initiate necrosis which has finally spread to the whole loop. The Allis's forceps seen in the photograph have been applied to the skin edges merely/



merely for the purpose of illustration. The lateral cut edges are united behind the loop. Great care must be taken when suturing the angles of the tube; no tension must be present and the sutures must include the deep fascia as well as the skin. In the six otherwise successful cases a fatal haemorrhage occurred from the angle of the loop due to the artery, which was lying immediately under the line of suture, having become adherent to a small scab, which, on separation, caused a breach in the vessel, - hence the necessity for ensuring that a layer of subcutaneous tissue separates the artery from the suture line.

In carrying out this operation the results have steadily improved, not due to any radical alteration in technique, but due to careful selection of animals, rigid attention to detail, especially as regards asepsis, and to increasing skill. Up till March 1935 one hundred rabbits had been used for constructing simple carotid loops; of these, four died before the operation had commenced, five died during the operation, and fifty successful loops resulted from the remaining ninety-one rabbits. During the period March 1935 till January 1937 one hundred/

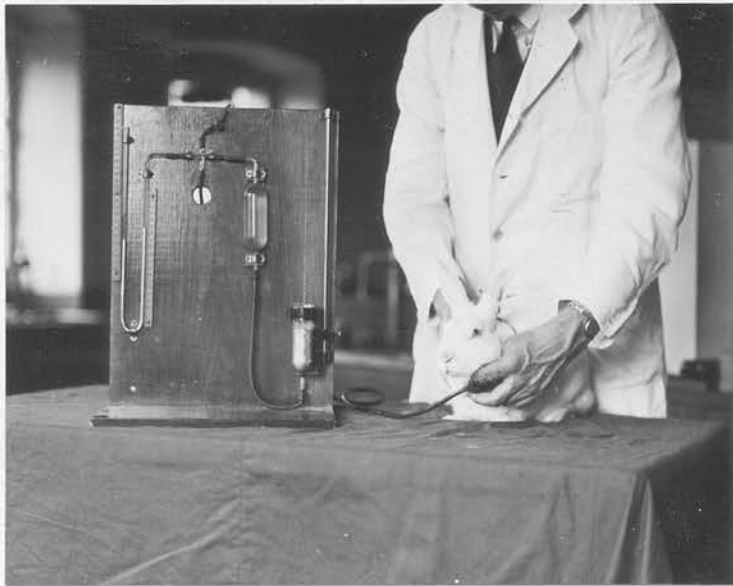
hundred and fifty-six animals were used; five died before or during the operation and from the remaining one hundred and fifty-one rabbits there resulted one hundred and seventeen successful carotid loops. It will be seen, therefore, that the results improved from 50 per cent to 75 per cent. These results compare favourably with those of other workers in this field. Failure in the vast majority of cases was due to dry necrosis of the overlying loop of skin associated with thrombosis of the artery. It was early discovered that all the animals had to be provided with round-edged feeding dishes, and that care had to be taken to ensure that the cages were free from sharp projections, otherwise they were very liable to tear their loops. A further risk is attendant upon pregnancy; six of the successfully looped animals tore them with fatal results. The apparent cause was that they caught the loop during the course of plucking their fur for the purpose of making a nest for the young. Several successfully looped animals died of intercurrent infections, such as tuberculosis, dysentery, and snuffles.

(8) It/

It will be seen, therefore, that the method carries with it many risks and is apt to be disheartening, especially at the outset. However, it is such a successful and reliable method for recording pressure that we consider the difficulty and expense fully justified.

We consider that strict adherence to the following rules will materially increase the percentage of successes:-

- (1) Only well-grown, healthy chinchilla rabbits of a minimum weight of 2 kgms. should be used.
- (2) No depilating preparation should be used on the skin.
- (3) Strict asepsis.
- (4) As much subcutaneous tissue as possible should be included in the skin loop.
- (5) The skin of the loop should be handled with great care and no forceps should be clamped on the skin edges.
- (6) Great care should be taken to ensure arrest of all haemorrhages before suturing the wound.
- (7) Careful suturing at the end of the loop and the avoidance of tension.
- (8) Frequent inspection of the wound for the first few/



Recording Blood Pressure by Carotid Loop Method.

Manual Apparatus.



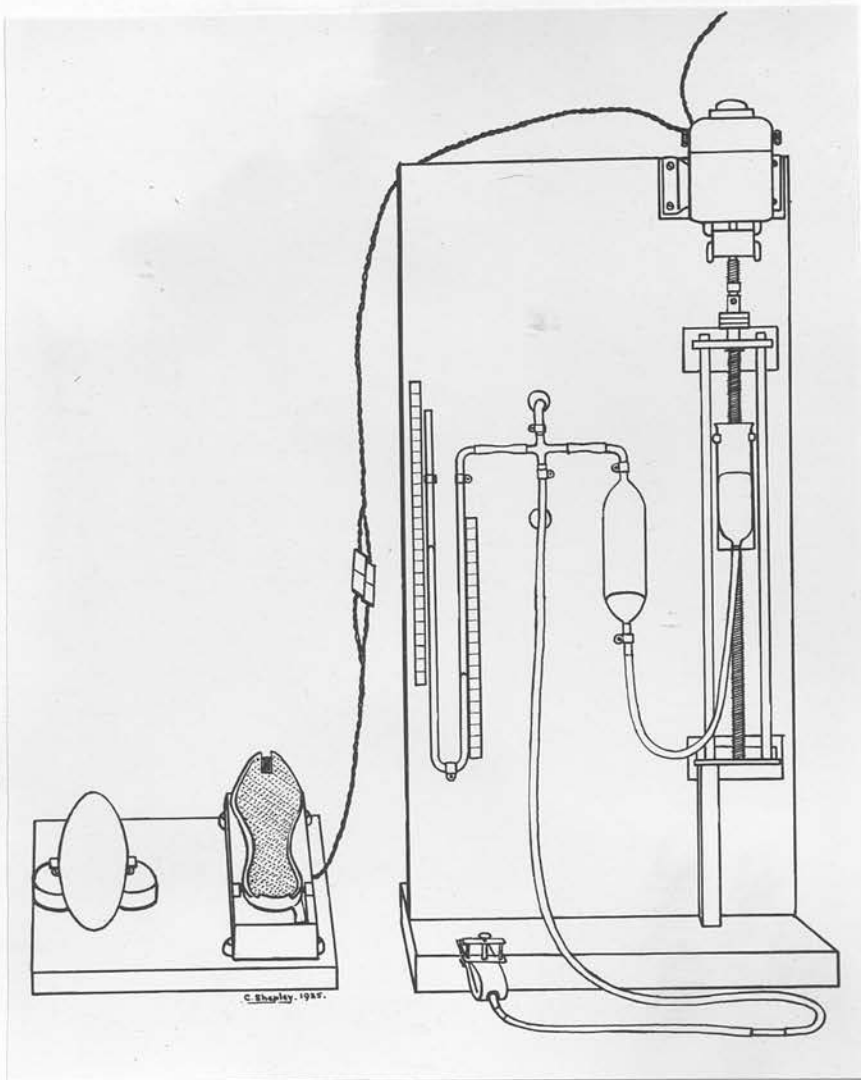
few days. If there is any trace of infection the wound should be swabbed with hydrogen peroxide, dried, swabbed with iodine and smeared with sterile vaseline.

(9) The animal should be kept away from all sharp edges.

(10) Pregnancy should be avoided.

Apparatus for Estimating Blood Pressure: The apparatus used was a slightly modified form of that used by Kremer, Wright and Scarff (1933). There is a small rubber bag encased in chamois leather. Outside this bag there is a U-shaped piece of flexible copper strip; by means of a clip the strip with the bag can be secured round the carotid loop. The bag is filled with water and it communicates by rubber tubing with a bulb filled with mercury. The level of the mercury in this bulb can be altered by means of an adjustable reservoir, and so the pressure in the bag can be raised or lowered. By means of a side tube the bulb communicates with a mercury manometer and so the pressure in the bag can be read directly.

After the first few determinations the rabbits become quite accustomed to the procedure and remain still/



Electrically Driven Blood Pressure Recorder.

still. Pressure is raised in the bag until the artery is no longer felt to pulsate distally; that point is taken as the systolic pressure. Six readings are taken on each occasion and it is found that the last four are almost invariably in very close agreement. Frequently all readings are identical. In order to ensure accuracy the readings are taken at approximately the same time each day (09.30 hours and 16.00 hours). As far as possible it is always the same operator who palpates the vessel.

Power-driven Blood Pressure Recorder: The fact that the apparatus just described always required two operators was found to be very inconvenient, especially when it became necessary to observe pressures hourly for 24 hour periods. A modification was accordingly made in order to enable one person to control the level of the mercury while retaining both hands free to hold the rabbit and to palpate the loop.

A screwed rod was substituted for the vertical slide rod on which travels the mercury reservoir. The upper extremity of this rod was directly coupled to the driving spindle of a Klaxon geared unit, comprising/

comprising a totally enclosed spur reduction gearbox giving a final speed of 1500 R.P.M. at a torque of 1 lb./inch, together with a series wound direct current motor running at 230 volts, fitted with split fields for electrical reversing. The motor is controlled by a foot-operated control panel comprising a rheostat and reversing switch. After many thousands of observations this apparatus has been found entirely satisfactory.

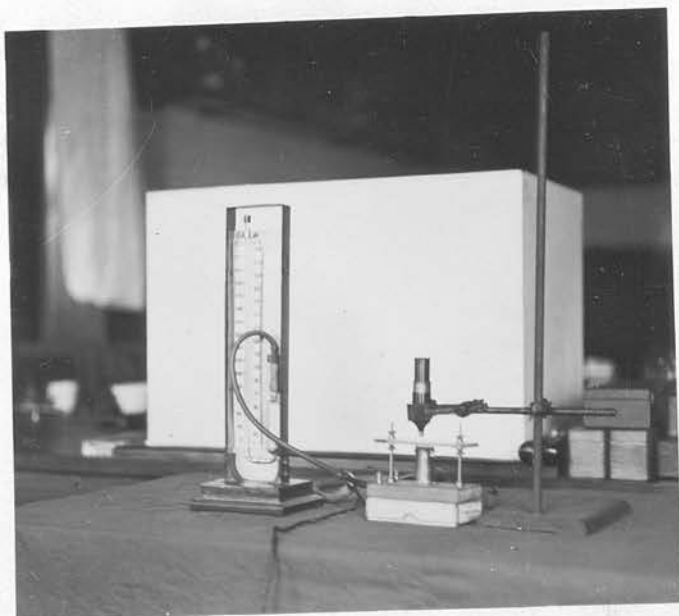
One has a greater belief in the accuracy of this method than in the recording of pressure in man with the arm-band, because there is little tissue other than the artery itself to compress.

The Normal Blood Pressure in the Rabbit: Our observations show the average pressure to be 95 mm. Hg. with a normal range of 65 to 125 mm. Hg. This agrees well with the figures of Tigerstedt who found the mean systolic pressure in the rabbit to be 100 mm. Hg. with a range of 80 to 120 mm. Koch and Koller analysed the readings obtained in 63 animals in the laboratory at Cologne under a variety of acute conditions and under different forms of anaesthesia. The results fell along a very smooth distribution curve with a mean pressure of 95 mm. and/

and a range of 65 to 125 mm. Van Leersum himself using the loop method gives the normal range as 70 to 140 mm. Scarff, using the same method, found an average pressure of 95 mm.

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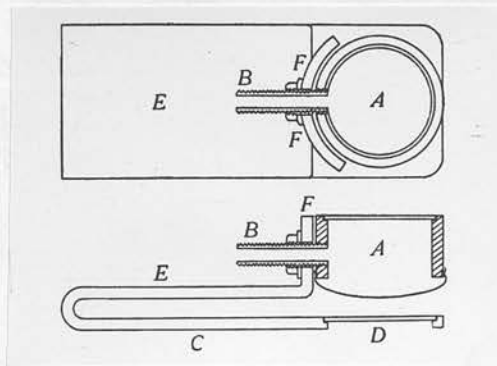


Recording Blood Pressure by Ear Artery.

Modified Apparatus of Squier.

Personal Experience of Other Methods  
of Recording Blood Pressure in the Rabbit.

Central Artery of the Ear: An apparatus for this purpose was constructed according to the specification of Squier (1927). However, it was soon found that the diameter of the central tube was far too large and so the apparatus was remodelled with a tube of 2 cms. diameter. The vessel was inspected through a very low power botanical microscope. Considerable difficulty was usually experienced in getting the rabbit's ear adjusted in the apparatus. The end point was found to be quite sharp. The mean pressure was 70 mm. Hg. which is 30 mm. lower than that obtained by the carotid loop method and by acute experiments. This in itself would seem to indicate that the central artery of the ear is fairly distal in the arterial system. An acute experiment was carried out. A cannula was introduced into the left carotid artery, the rabbit being under urethane anaesthesia. The carotid pressure was recorded with a kymograph. Pressure was recorded in the right ear at intervals of about one minute; the instant of disappearance of pulsation/

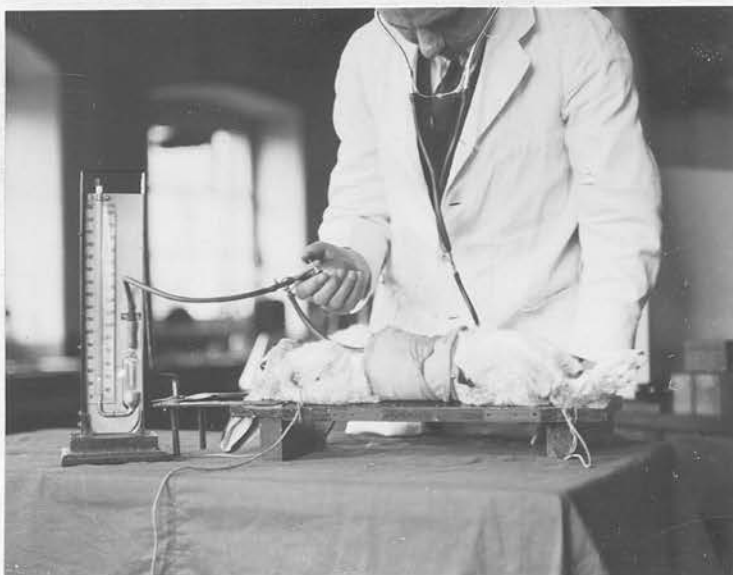


Ear Artery Capsule of Grant & Rothschild.

pulsation in the artery was recorded by an electric signal on the moving paper below the kymographic tracing. During a quiet period the pressure in the central artery ran parallel to the carotid pressure. However, when a small dose of adrenalin hydrochloride was given intravenously the central artery of the ear literally vanished while the carotid pressure rose abruptly. It was not until several minutes had elapsed that it was possible to once again record the ear pressure.

This observation would seem to indicate the inherent unsuitability of the method; however, the recent paper by Grant and Rothschild (1934) seems to indicate satisfaction with the procedure. Their apparatus is very light and appears to get over many of the mechanical difficulties of Squier's apparatus. A model according to their specification has been constructed and with it numerous observations were made but these did not bear a constant relation to the carotid pressures.

One theoretical consideration appears important. According to the principal law of hydrodynamic pressure enunciated by Poisseuille the pressure in a tube varies inversely as the fourth power of the radius/



Recording Blood Pressure by Abdominal Band Method.



radius of the tube.

$$P = \frac{8nLQ}{\pi R^4}$$

where

Q is the volume of the flow per unit of time.

P is the pressure.

R is the radius of the tube.

n is the coefficient of viscosity.

L, the length of the tube.

Now Grant and Rothschild state that there may be a ten-fold variation of the radius of the central ear artery as the result of cooling. One has noticed complete disappearance of the artery under adrenalin stimulation. Therefore, no other conclusion appears possible than that this artery is subject to purely local variable factors which are by no means entirely eliminated by previous denervation. Consequently it would appear to be unsuitable for the purpose of gaining a true index of systemic pressure.

#### The Abdominal Band Method of MacGregor.

Several observations were carried out by this method but the systolic pressure recorded appeared to be at least 20 mm. higher than the carotid loop pressure. Indeed, a perusal of the curves published by/

by MacGregor himself shows that the mere compression of the abdomen is sufficient to cause a reflex rise of pressure of at least 20 mm. Hg. The procedure caused the rabbit considerable discomfort. One definite advantage, however, is that it is possible to record diastolic pressure by this method.

WILSON: *Trans. Roy. Soc. Can.*, 1927, 20, 57.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 305.

WILSON & LEVY: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Jour. Metabol. Research*, 1924, 2, 143.

WILSON: *Reports Johns Hopkins Hosp.*, 1906, 3, 55.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 314.

WILSON & LEVY: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON & LEVY: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON & LEVY: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON: *Can. Jour. Physiol. & Pharmacol.*, 1928, 6, 311.

WILSON:

R E F E R E N C E S.

- ALLEN: Jour. Metabol. Research, 1923, 4, 431.
- ANDERSON: Proc. Soc. Exp. Bio. & Med., 1922-23, 20,  
295.
- BACKMANN: Compt. rend. Soc. de Biol., 1916, 79, 338.
- BEHRENS: Arch. f. d. ges. Physiol., 1926, 212, 372.
- BIASOTTI: Compt. rend. Soc. de Biol., 1927, 97, 597.
- BRIGHT: Guy's Hosp. Reports, 1826, 1, 338.
- COHN & LEVY: Jour. Exp. Med., 1920, 32, 351.
- DOMINGUEZ: Jour. Metabol. Research, 1924, 6, 123.
- ERLANGER: Reports Johns Hopkins Hosp., 1904, 12, 53.
- FAHR: Verhandl. d. deutsch. Path. Gesellsch., 1912,  
15, 234.
- FERRIS & HYNES: Jour. Lab. & Clin. Med., 1930-31, 16,  
597.
- GÄRTNER: Wien. Med. Wchnschr., 1899, 30, 1411.
- GOLLA & ANTONOVITCH: Quart. Jour. Med., 1930, 23, 167.
- GOTZE: Berl. Tierärztl. Wchnschr., 1920, 26, 293.  
(Consulted in abstract)
- GRANT. & BLAND: Heart, 1933, 16, 69.
- GRANT & ROTHSCCHILD: Jour. Physiol., 1934, 81, 265.
- GRIFFITHS: Proc. Soc. Exp. Biol. & Med., 1934-35,  
32, 394.
- HALES: Quoted by Starling "Human Physiology".
- HANZAL, GOLDBLATT, LYNCH, & SOMERVILLE: Jour. Exp.  
Med., 1934, 59, 347.
- HARTMANN, BOLLIGER, & DOUB: Amer. Jour. Med. Sci.,  
1926, 172, 487.
- HÜRTHLE: Arch. f. d. ges. Physiol., 1890, 47, 1.
- JANEWAY/

- JANEWAY: "The Clinical Study of Blood Pressure."  
1904, 31.
- JANEWAY & PARK: Proc. Soc. Exp. Biol. & Med., 1907-10,  
5, 156.
- KOCH & KOLLER: Ztschr. Bio., 1932, 92, 202.
- KOCH & MIES: Ztschr. f. d. ges. exp. Med., 1928,  
62, 551.
- KOLLS. Jour. Pharmacol. & Exp. Therap., 1920, 15,  
443.
- KOTTLORS & ROTHSCCHILD: Ztschr. f.d.ges. exp. Med.,  
1932, 84, 795.
- KREMER, WRIGHT, & SCARFF: Brit. Jour. Exp. Path.,  
1933, 14, 281.
- KUNSTMANN; Arch. f. exp. path. u. Pharmakol., 1928,  
133, 122.
- KURAYA: Acta Scholae. Med. Univ. Imp. Kioto., 1924,  
6, 375.
- LUDWIG: Arch. f. Anat. Physiol. u. Wissench. Med.,  
1847, 242. ( Consulted in abstract.)
- MCGREGOR: Arch. Path., 1928, 5, 630. (Includes a  
most valuable review.)
- MAEKAWA: Acta Scholae. Med. Univ. Imp. Kioto,  
1930, 12, 341.
- MARTIN: Münch. med. Wehnschr., 1903, 50, 1021.
- NEUMANN: Klin. Wehnschr., 1930, 9, 695.
- PAGE: Amer. Jour. Physiol., 1935, 112, 166.
- PETROFF: Arch. f. d. ges. Physiol., 1929, 223, 477.
- POISEUILLE: Quoted by Tigerstedt: Physiologie des  
Kreislaufes 1, 104, & 3, 143.
- RAI: Acta Scholae Med. Univ. Imp. Kioto, 1930-31,  
13, 245.
- SCARFF: Jour. Path. & Bact., 1927, 30, 747.
- SCHLESINGER/

- SCHLESINGER: Arch. f. exp. path. u. Pharmakol.,  
1930, 149, 317.
- SCHMIDT-WEYLAND: Ztschr. f. d. ges. exp. Med.,  
1931-32, 80, 341.
- SCHÖNHEIMER: Arch. f. path. Anat., 1924, 249, 1.
- SHAPIRO, SHEPHARD, & SEECOF: Jour. Lab. & Clin. Med.,  
1924-25, 10, 826.
- SQUIER: Jour. Lab. & Clin. Med., 1927, 13, 279.
- TIGERSTEDT: "Physiologie des Kreislaufes." 1911,  
142, 377.
- TRENDELENBURGH: Ztschr. f. d. ges. exp. Med., 1913,  
2, 1.
- VAN EWEYCK & SCHMIDTMANN: Arch. f. path. Anat.,  
1922, 236, 421.
- VON DEICKE: Krankh. Forschung., 1926, 3, 399.
- VAN LEERSUM: Arch. f. Physiol., 1911, 142, 377.
- VON RECKLINGHAUSEN: Arch. f. exp. Path. u. Pharmakol.,  
1906, 55, 375.
- WATSON: Jour. Lab. & Clin. Med., 1927, 12, 706.
- observations showing the presence of adrenal hyper-  
plasia of the suprarenals in cases of hypertensive  
nephritis. This was hailed as a further support of  
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morphological investigations, particularly those  
of Pearce (1905), Thomas (1910), and Jordan (1907)  
have failed to show a constant relationship between  
suprarenal disease and hypertension. Furthermore,  
suprarenal adenomata have frequently been observed  
in/



Possible "Pressor-body" Origin of Hypertension.

The basis of the many chemical theories of hypertension undoubtedly lies in Bright's suggestion that altered composition of the blood is the probable cause.

It is hardly surprising that the discovery by Oliver and Schäfer (1895) of pressor extracts of the suprarenal gland should be hailed as the solution of the chemical origin of hypertension. The hypothesis that hyper-activity of the suprarenals might be the cause of nephritic hypertension was suggested by Neusser (1898) who had observed a case of chronic Bright's disease, associated with carcinoma of one suprarenal. Vaquez (1904) reported certain autopsy observations showing the presence of nodular hyperplasia of the suprarenals in cases of hypertensive nephritis. This was hailed as a further support of the adrenal origin of hypertension but further morbid anatomical investigations, particularly those of Pearce (1908), Thomas (1910), and Landau (1907) have failed to show a constant relationship between suprarenal disease and hypertension. Furthermore, suprarenal adenomata have frequently been observed in/

in cases free from hypertension. Experimental lesions produced by adrenalin have been found to be quite unlike human arteriosclerosis. Ingier and Schmorl (1911) determined the adrenalin content of the suprarenals in a series of 517 autopsies. They found an increase in acute nephritis and in 17 patients with chronic nephritis, with higher values, on an average, in those having high blood pressure and hypertrophied hearts. However, their four highest figures were from a case showing neither cardiac hypertrophy nor arterio-sclerosis. Further, the case showing the greatest cardiac hypertrophy showed a subnormal content of adrenalin.

The whole picture of Addison's disease with its hypotension was assumed to be the opposite syndrome to hypertension. However, with further detailed and precise experimental work, specially that of Hoskins and McClure (1912), it has become apparent that the operative removal of the suprarenals is not necessarily followed by immediate hypotension, and the effect of adrenalin is so transient that, with the normal tonus of the blood vessels dependent upon its presence, the removal of the suprarenals must inevitably cause a prompt and unmistakable/

unmistakable fall in blood pressure. The modern tendency is to regard the cortex of the suprarenal as concerned principally with the maintenance of resting blood pressure, while the medulla with its secretion of adrenalin constitutes a mechanism for the elevation of pressure in time of emergency. A considerable advance was made when it became possible to estimate to an extreme degree of accuracy the quantity of adrenalin present in solution. Schur and Weisel (1907) claimed to have demonstrated the presence of adrenalin in the circulating blood of nephritics. They used as their test object the excised eye of the frog, which dilates with adrenalin. This report was rapidly followed by that of Schlager (1907) who made tests by the Meyer artery strip method, using normal sera and, in ten experiments, nephritic sera from hypertensive patients. It was found that normal serum after the removal of albumin always contained a constrictor substance which resisted dialysis and was diminished by concentration in vacuo and therefore resembled adrenalin. Nephritic sera in all cases produced much less constriction than normal sera. He concluded that he had been unable to demonstrate any/

any excess of adrenalin-like substance in the blood of cases of nephritic hypertension.

A series of papers by Stewart and Zücker (1911-13) dealt a severe blow to the hypothesis that the maintenance of the normal vascular tone in the intact animal and in man must be maintained by the constant secretion of adrenalin into the blood. He called attention to the fact which most of the clinical investigators had disregarded, that the property of stimulating arterial muscle or pregnant uterine muscle is not peculiar to adrenalin. He suggested that the manipulation of the blood after withdrawal might be associated with the release of bodies with an adrenalin-like action. O'Connor (1912) published a report which appeared to show that the vaso-constrictor substance in defibrinated blood and serum which had been mistaken for adrenalin was equally stimulating to intestinal muscle and could not, therefore, be adrenalin. He furthermore proved that the constrictor effect could be abolished by the careful prevention of coagulation by the addition of either citrate or hirudin. This confirmed Stewart's contention that the pressor body was released during the process of coagulation. About/

About the same time Janeway and Park (1912), as the result of a long series of experiments made by a modified Meyer method, had already reached the conclusion that the vaso-constrictor substance of defibrinated blood was not adrenalin. They used as their test objects rings of the carotid artery of the ox which are normally constricted by adrenalin, and rings of the coronary arteries which are normally dilated. Defibrinated blood and serum produced extreme constriction of both preparations. In general the character of the curves obtained bore no resemblance to those obtained from adrenalin stimulation. It appeared that the serum constrictor substance acted upon smooth muscle directly and not upon its sympathetic innervation. In further work by the same authors they were unable to determine any difference in pressor effect between blood from normal patients and in blood from hypertensive patients. Further work by Hoskins and McClure (1912) showed that the quantity of adrenalin necessary to produce a minimal rise in blood pressure is from ten to twenty times the amount normally secreted. As they have further shown that the injections of an amount sufficient to/



to raise blood pressure caused a complete inhibition of intestinal peristalsis, they argue that adrenalin could not be a direct factor in the maintenance of the normal tonus of the vaso-motor system. They considered that the normal adrenalin concentration of arterial and venous blood is in the region of 1 in 200,000,000, an amount quite undetectable by the most delicate methods. One could quote many other observations, the vast majority of which tend to show that adrenalin plays little, if any, part in the normal maintenance of blood pressure. Furthermore, except for isolated cases of tumours of the adrenal medulla it plays no part in pathological hypertension.

The hypothesis that the pressor body of the pituitary causes vascular hypertension may be said to date from 1908 when Herring demonstrated histologically the secretion globules in the posterior part of the pituitary. Two years later Cushing and Goetsch (1910-11) claimed to have demonstrated in the cerebro-spinal fluid substances which behaved in such a fashion as to indicate their probable pituitary origin. Since that time there has been much contradictory work on this question, with confirmation/

confirmation by some workers and refutation by others. Recently, Hoyle (1933) entirely failed to demonstrate pituitrin in the cerebro-spinal fluid either of normal people or of cases suffering from nephritis or essential hypertension. That Cushing (1933) still believes in the pituitary origin of certain hypertensive states is evident from his recent publication in which he claimed to have demonstrated cases of the basophil invasion of the posterior lobe of the pituitary in certain cases of eclamptic toxæmia. The most recent piece of work in which it is claimed that the influence of pituitary has been demonstrated in the blood is that of Anselmino, Hoffman and Kennedy (1931) which will be described later.

Major (1925-27) and his co-workers have repeatedly suggested that essential hypertension may be due to guanidine bases in the blood which have been retained as the result of renal damage. The most fundamental criticism of his work is that the chemical methods available are not delicate enough to differentiate between the guanidine and certain allied bodies similar to creatinine. In particular, Greenwald (1924) and White (1927) do not believe that/

that there is satisfactory evidence that guanidine bases are actually excreted in normal urine.

Numerous attempts have been made to determine the presence or otherwise of pressor bodies in blood by the injection into animals of either whole blood, alcoholic extracts or ultra-filtrates of blood. Danzer and Miles (1925) reported a series of experiments which, according to them, showed that blood from cases of hypertension contained a pressor substance. They introduced 12 - 20 cc. of whole blood directly into the veins of cats. The result was an immediate pressor effect followed by a depressor effect. Repeated injections in the same animal during acute experiment resulted in the progressive diminution of the depressor response which they therefore concluded to be an allergic phenomenon which could be destroyed by desensitization. They also tried an alcoholic extract of hypertensive blood and found it to have a depressor effect. They further state that no pressor effect was ever observed following the injection of comparable amounts of saline, rabbits' blood or human blood from people with normal blood pressure. Two years later Curtis, Moncrieff and Wright (1927) concluded/

concluded that there was no evidence of the presence of a pressor substance in the blood of patients with hypertension. They used cats anaesthetised initially with ether, then with intravenous chloralose. They found that after the animals had been completely desensitised to human blood neither normal nor hypertensive blood caused any elevation of pressure. They suggested that the pressor effect obtained by Danzer, Brodie and Miles was due to incomplete desensitisation of their animals.

Georghian and Niculescu (1924), using injections of 5cc. of serum from hypertensive patients found that the blood pressure of the rabbit was uniformly raised by as much as 28 mm. Hg., whereas similar quantities from normal subjects showed no rise. Elliot and Nazum (1932-33) injected citrated whole blood of normal and hypertensive patients, intravenously, into rabbits in doses of 3 cc. per Kgm. In most instances they noted that a transitory rise in pressure was followed by a prolonged fall. They formed the conclusion that the average initial rise in pressure of the animals receiving blood from patients with hypertension was slightly greater and more prolonged than that noted in the control series. The/

The alcohol, water and ether soluble fractions of such blood gave a uniformly transitory fall in pressure. However, they are careful to point out that their results are not statistically significant and are to be regarded as inconclusive.

Volhard (1923) apparently believes that renal hypertension is due to the action of a pressor substance in the blood. His pupils, Hülse and Strauss (1924) claim to have demonstrated in the blood of patients with glomerulo-nephritis and eclampsia a substance which sensitises the arterioles to adrenalin and thereby produces arteriolar spasm. At first they considered the sensitising substance to be of the nature of peptone, but more recently Hülse and Franke (1924) have considered that it is an amino body of the type of choline.

One of the most extensive research reports emanating from Frankfurt a. Main was that of Bohn (1931-32). He prepared alcoholic extracts of blood and tested them on curarised cats, anaesthetised with ethyl urethane. He concluded that a pressor substance was present in the blood of patients suffering from nephritic hypertension, from eclampsia, and from "nephro-sclerosis maligna".

On/



On the other hand, the blood of normal subjects or cases of essential hypertension contained no pressor body. The substance was found to be ultra-filtrable and rendered inactive by exposure to ultra-violet light or to alkali. It also acted as an anti-diuretic, and Bohn believed the substance to be pituitrin. These experiments were taken as substantiating Volhard's hypothesis that the mechanism of nephritic and essential hypertension is fundamentally different, the former depending on the presence of a chemical substance, and the latter being predominantly of nervous origin.

Marx and Hefke (1933) found that alcoholic extracts of blood from nephritic patients with hypertension induced a prolonged rise of blood pressure in non-anaesthetised dogs. On the other hand, he found that ultrafiltrates were inactive. In this respect he differed from Bohn.

Anselmino and Hoffmann have advanced a view that hyperfunction of the posterior lobe of the hypophysis is the fundamental defect in eclampsia and nephropathy of pregnancy. They claim to have isolated from the blood of cases of eclamptic toxaemia/

toxaemia a pressor and antidiuretic substance. Their method was to withdraw from the patient 40 cc. of venous blood, using as anti-coagulant 2 cc. of 5 per cent sodium citrate. The blood was centrifuged and to it was added 1 cc. of N. acetic acid per 20 cc. of plasma, thus bringing the pH to the neighbourhood of 4. The plasma was then filtered under a negative pressure through an acetic collodian filter. The ultra filtrate was protein free and it was kept on ice until required. Immediately prior to use it was neutralised with NaOH. As a test for the anti-diuretic activity of the extract they employed the method of Kestranek, Molitor and Pick (1915) which consisted of giving fasting rabbits 90 cc. of water by stomach tube and administering subcutaneously 10 cc. of ultra filtrate, or in the case of the controls, 10 cc. of N. saline, plus a suitable quantity of a neutral acetic acetate mixture. Urine was obtained from the animals at half hour intervals by pressing the bladder, and the chloride content of each sample was analysed. In the control animals it was found that urinary secretion increased up to the third or/

or fourth half hour, giving an increasingly low content of chloride. The subcutaneous injection of a small quantity of pituitrin inhibited this diuresis, resulting frequently in anuria during the third half hour period. The urine in these cases showed a correspondingly high chloride content. It was claimed that similar inhibition of diuresis was obtained by the injection of the plasma ultra-filtrate, similar curves being obtained from all of 24 cases of toxic albuminuria and eclampsia which showed oedema clinically. They found that ultra-filtrates, known to be antidiuretic, were inactivated by alkalisation, exposure to ultra-violet light for an hour, or shaking with talc. They considered that the results indicated that pituitrin was responsible for the action of the ultra-filtrates. The pressor activity of the ultra-filtrates was observed by the method of Koch and Mies on unanaesthetised animals (method described elsewhere) and also by means of the usual acute experiment method. They claimed that there was an elevation of pressure of about 25 mm. Hg. following the injection of 12 cc. of ultra-filtrate. A further injection produced a less pronounced pressor response, resembling/

resembling in that respect the action of pituitrin. They state that the pressor substance was recognisable in 10 - 12 cc. of all cases of eclamptic toxæmia in which the B.P. of the patient reached 180 mm. Hg., whereas it was never observed from as much as double the quantity of ultra-filtrate obtained from healthy pregnant and non-pregnant women. It showed the same susceptibilities as did the anti-diuretic principle.

This apparent proof of the posterior pituitary origin of the oedema and hypertension of eclampsia attracted much attention, but it was apparently not until 1933 that any serious attempt was made to repeat the work, when Byrom and Wilson (1934) made further observations on the possible presence of pituitary extract in the blood of cases of eclamptic toxæmia. They commenced by considering the suitability of the rabbit as a test animal for the presence of an anti-diuretic factor, and in their hands they found the rabbit method useless. They observed that the normal range of hydration in the fasting rabbit was so wide that 100 cc. of water often failed to cause diuresis. Further, they found that when extra water was left in the cage overnight/

night the rabbit often imbibed so freely that either diarrhoea resulted, or the experiment was begun on an animal which was already showing a brisk diuresis. In addition, they found the frequent contamination of the urine with mucus rendered difficult both measurement of volume and analysis. They therefore substituted Burn's method for the assay of the anti-diuretic principle of pituitary extracts. This consisted of the introduction of water into the stomach of a fasting rat in the proportion of 5 cc. per gram-body-weight. Immediately afterwards a measured volume ( 1 cc. per hundred grams weight) of the suspected ultra-filtrate was injected subcutaneously. Three other rats were treated in the same way with as little delay as possible. Four animals were placed in a metabolism cage resting on a large glass funnel which drained into a 25 cc. cylinder. A second group of rats was similarly treated, using either control ultra-filtrate prepared from normal blood or normal saline. The total volume of urine excreted was measured every fifteen minutes until diuresis had subsided. After an interval of one clear day the experiment was repeated, but the two groups of rats were reversed. When/



When the amount of available ultra-filtrate permitted, several groups of rats were used. In rats which received no pituitrin the peak of the diuresis occurred within 50 - 60 minutes after the introduction of water into the stomach. If a filtrate containing pituitrin was used the onset of diuresis was delayed and its peak postponed beyond the normal limit. Burn states that the smallest amount of pituitrin which can be consistently demonstrated by this technique is about .001 International Units per 100 grams of rat. Byrom and Wilson (1933) first carried out experiments to ascertain whether the method was sufficiently sensitive for their purpose. They added known quantities of pituitrin (P.D. & Co.) to ultra-filtrates. They found that the method was sensitive enough to detect pituitrin in ultra-filtrates of plasma containing .002 units per cc., i.e. in the minimum concentration which was alleged by Anselmino and Hoffman to occur in the mildest cases of pre-eclamptic toxæmia.

In selecting cases for investigation the following clinical manifestations were considered essential:- (1) Albuminuria; (2) Visible pitting oedema/

oedema; (3) A systolic B.P. of between 140 and 190 Hg. Their series included three cases of frank eclampsia. Ultra-filtrates were made from 13 cases of toxæmia and 38 observations were made, each based on 4 rats. These ultra-filtrates had no effect on normal diuresis of the rats. Extensive control observations were carried out and none of these showed any effect except when pituitrin was intentionally added to the ultra-filtrate. This research has apparently been carried out with great care, and although the number of cases observed is small the results were consistently negative. Their summary may be quoted verbatim, - "A method which is capable of detecting the anti-diuretic hormone of the pituitary after addition to blood plasma of a dilution of 0.002 International Units per cc. has failed to reveal the presence of the hormone in ultra-filtrates derived from the plasma of 10 cases of pre-eclamptic toxæmia of pregnancy and 3 cases of eclampsia with oedema". The nature of this contribution is such as to suggest that in one major aspect the work of Anselmino and Hoffman is incorrect. There is little reason to regard their work on the "pressor-body" content of eclamptic blood as any more accurate.

De/

De Wesselow and Griffiths (1934) published an account of certain investigations into the question of pressor bodies in the blood of hypertensives. They utilised 40 cc. of citrated blood. This was precipitated with 9 volumes of absolute alcohol, shaken and allowed to stand for 5 hours on ice and then filtered. The filtrate was evaporated in vacuo at 40 C to 20 cc. and then re-precipitated with 2 volumes of alcohol. The filtrate from this precipitation was evaporated in vacuo at 40°C to a few cc. and made up with saline to 5 cc. before injection. For plasma the same process is employed except that only 2 volumes of alcohol were used and the alcoholic extract was not re-precipitated. Extracts obtained showed only a faint trace of protein and their pH was approximately 8.5. They also employed extracts prepared by the ultra-filtration method suggested by Hoffman and Anselmino. In some cases they employed Bohn's method which consisted of precipitating plasma with alcohol after an initial acidification to 4.5 with acetic acid. They discussed the very important question of dosage of the extract, pointing out that, using cats of an average weight of about 3 Kgm. they at the most only produced/

produced concentration of the substance in the cats' blood equivalent to 1/10 of that in the patient's blood. Thus it is not to be expected that any hypertension due to pressor bodies would be of a pronounced degree. In testing the extracts they used tracheotomised cats under urethane anaesthesia. They allowed some time to elapse between the operative procedure and the start of the injections in order to be certain that the blood pressure was at a constant level. Pressure was recorded in the common carotid and the injections were made into the femoral vein. Extracts of ultra-filtrates were invariably made up to 5 cc. before injection. The sensitivity of the animal to pressor bodies was tested by adrenalin injection. As far as possible the same animal was used for a normal as well as for a hypertensive blood.

They tested blood from cases of essential hypertension, so-called malignant hypertensives, chronic glomerulo-nephritics and cases of pre-eclamptic toxæmia. Their results were remarkably uniform, consisting of an initial fall of pressure which could be diminished by atropine. This fall was most marked with the whole blood extracts, while with/

with the plasma extracts it was slight or absent. It did not vary with the type of patient from which it was derived. Following the lowering of pressure there was a slight elevation of pressure which was more pronounced in the blood of essential hypertensives, although present to a lesser degree in all other extracts. Ultra-filtrates did not show this pressor effect. Their method of extraction resulted in a fluid which gave entirely negative results. In discussing their results they state that they are in direct disagreement with those of Bohn (1931-32). A pressor body is to be found in the alcoholic extracts of the whole blood of essential hypertensives, a type of blood which, according to Bohn, is destitute of pressor activity. On the other hand, they find no evidence that the depressor effect of alcoholic blood extracts, which Bohn believes to be characteristic of the blood of essential hypertensives and of normals, is so restricted. Their experience was that it was universally present. Furthermore, they find no suggestion that any variety of hypertension is due to the absence from the blood of a depressor body. They note further that pituitrin added to the blood/



blood or plasma is recoverable in the alcoholic extracts and also from the ultra-filtrates provided that the pH of the plasma prior to ultra-filtration is not brought to 4.5; when that was done pituitrin was not recovered. They found that adrenalin added to the plasma, which was then ultra-filtered, did not survive the process. They conclude by saying that they do not feel justified in assuming that with their methods they have obtained any evidence of pressor bodies in the blood of hypertensive patients.

A recent article by Page provides such a complete and careful account of pressor bodies in blood that no apology is needed for quoting it in some detail.

The extracts were prepared by centrifuging heparinised venous blood for three minutes and adding the plasma to 95 percent ethyl alcohol in the proportion of 10cc. plasma to 90 cc. alcohol. The flasks were then stored on ice for from 2 to 24 hours, the precipitated protein and lipid filtered off, and the clear filtrate concentrated to one half the original plasma volume. The alcohol was removed in a Claissen flask with water-cooled condenser/

condenser under 1 to 2 mm. vacuum. The temperature in the flask was not permitted to rise above 20 C. It is emphasised that all alcohol must be removed from the extract as it tends to accentuate the pressor effect of the extracts. Acetone extracts were found to be depressor in action, while ether extracts were inactive. Similarly, other precipitants were found to be unsuitable. It was further noted that the exact concentration of the alcohol used did not matter, provided that the protein precipitation was complete. Altering the pH to 4.5 by the addition of N acetic acid did not interfere with the efficiency of the alcohol extraction.

The resulting extract was protein-free as tested by salicylsulphonic acid. However, it contained a considerable amount of lipoid material, and although it was found that this did not interfere with the animal testing it was usually removed by cooling the extract to 8 C. and decanting the supernatant clear fluid. As regards the stability of the extract it was found that the duration of alcohol extraction in the refrigerator did not alter their pressor activity, but once the alcohol was removed/

removed they tended to lose this activity and to become progressively more depressor, especially if the extract was kept at room temperature.

The possibility of coagulation of the blood being a factor in the production of the pressor body was investigated. It was found that ascitic fluid contained as much pressor activity as blood. When such fluid was allowed to flow directly into alcohol it was found that its pressor activity was precisely similar to citrated or heparinised ascitic fluid.

The possibility of extraction by ultra-filtration was explored and it was found that the pressor body was not present in ultra-filtrates. Various methods were employed. Collodion sacs of varying porosity and pear-shaped porcelain thimbles, on which acetic acid collodion was deposited as a membrane, were tried. Ultra-filtrates were also prepared by high pressure (150 Kgms. per sq.cm. of nitrogen) filtration through membranes of varying density. The apparatus of Pfalz and Bauer was employed. This method had the advantage that 10 cc. or more fluid could be obtained in half an hour, the time depending on the porosity of the filter. Ultra/

Ultra-filtrates were also prepared from plasma at normal pH and at a pH of 4.5 obtained by the addition of acetic acid. Neutralisation of the acid dialysates (to phenolphthalein) was performed immediately before injection into the animal. The ultra-filtrates were protein free.

It was found that the pressor principle could be extracted from the alcohol extract with chloroform. The alcohol extract could be heated to 100 C for one minute with only partial loss of pressor activity, but more prolonged heating even at lower temperatures resulted in the progressive liberation of depressor bodies.

With regard to the question of whether the pressor body was associated with the lipoids it was found that the gross fats could be frozen out of the extracts without substantial loss of pressor activity. These fats in aqueous emulsion had no pressor activity. Further removal of phosphatide was achieved by precipitation with a large excess of acetone and allowing the precipitate to agglomerate at a temperature just above 0° C. The supernatant fluid, after the addition of water and the removal of/

of acetone under vacuum, was still vaso-pressor. It was therefore concluded that none of the ordinary lipoids were concerned with the pressor body.

Page made a careful study of the conditions under which the extracts were tested. He used non-pregnant cats of 3-4 Kgms. which had been deprived of food for 18 hours. Pressure was recorded in the usual manner by means of a cannula in the carotid or femoral artery. The injections of warmed extract were made into the cannulated femoral vein. The volume of these was approximately 5 cc. The cats were tracheotomised and anaesthetised by urethane in doses of 7 - 10 cc. of a 25 per cent solution, given 3 - 4 hours prior to the operation. Other anaesthetics were tried but urethane was found to be one of the most satisfactory.

It was early discovered that the re-activity of the cat to a pressor extract varied markedly during the course of the experiment. In order to avoid fallacy from this source an attempt was made to determine and classify the periods of the experiment according to the sensitivity of the cat. The importance of this was so crucial that a large number/



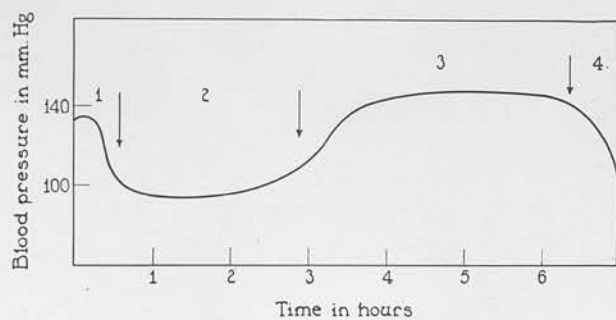


FIG. 1. Curve showing variations in blood pressure exhibited by a cat while anesthetized with ether.

Reproduced from Page's Article.

number of animals were employed. The accompanying copy of his diagram will serve to illustrate his results. During the first period the blood pressure is high and tends to fall rapidly and progressively; during this period pressor extracts have little effect. Indeed, they sometimes cause a depressor effect. The second period shows a constant lower pressure of one to three hours duration and maximum sensitivity to pressor extracts. This is followed by a third period of several hours duration, during which the pressure is somewhat higher and the animal's response to pressor extracts is sluggish. The fourth and final period is one of rapid deterioration marked by irregular pressure and asphyxial spasms. Extracts were always tested during the second period, and when comparing them with controls the tests were performed within the shortest possible period and in alternating order.

Attempts were made to estimate the reactivity of animals to pressor bodies by preliminary exhibition of such substances as adrenalin, pitressin, choline, adenylic acid, histamine. These peripherally-acting agents gave no prediction of the response/

response to a pressor extract, which observation can be correlated with the fact that the pressor bodies of blood would appear to act centrally. A reliable index of sensitivity was found to be the pressor response to the inhalation of a CO<sub>2</sub> air mixture. Although parallelism between the elevation of blood pressure due to this mixture and that due to the pressor extract was not exact it served to detect any serious variation in the sensitivity of the animal.

Among the other precautions observed were:-

(1) Estimation of effect on the pressure, of the intravenous injection of an equivalent volume of warm saline. (2) Observation of the effect of the injection of equivalent volumes of fluid containing urea and sodium chloride in the same proportions as in the extracts. No effect was noted on the pressure.

Page found that a typical pressor response to the plasma extracts consisted of a slow rise in the level of the blood pressure and the steady maintenance of the elevated level for some time. There was no initial depression. He tested combinations of extract with various drugs. With ergotoxine/

ergotoxine there was some reduction of the pressor effect, especially after large doses when the central nervous system was obviously much depressed. In the same animal there was at the same time the characteristic reversal of the adrenalin pressor effect. The effect of the extract in an animal which had been subjected to cocaine was observed. It was found that although there was much increased sensitivity to adrenalin there was no increased sensitivity to the extract.

Atropinisation to the extent of reversal of choline action did not alter the extract response.

The effect of the extract on kidney volume was observed. In general it was found that active plasma extracts tended to cause the renal volume to increase slightly, although there was extreme variability in the renal volume response. In parenthesis it may be noted that Stewart and Zücker (1912) claimed that they had found in plasma a protein of the albumin class which caused the direct vaso-dilatation of the renal vessels. Page, however, was dealing with protein-free extracts.

In an attempt to find which part of the vascular system was responsible for the elevation of pressure/

pressure Page observed variations of volume of a limb. He found in sixteen animals a moderate constriction in leg volume following the injection of plasma extracts. Further, he observed the effects of the pressor extract in eviscerated animals and his results were such as to suggest that constriction of the vessels of the splanchnic area played a large part in the pressure elevation following the injection of the plasma extract. In order further to eliminate the possibility of hyperadrenalism as the agent of the pressor response, Page observed the effect of the extract in bilaterally adrenalectomised cats. There was no diminution in the pressor response.

The question of whether or not immunity could be developed to the pressor action was considered. It was found that repeated injection of the extract augmented the pressor response until levels above 160 mm. Hg. were attained. Above this, further increase could not be obtained. This, however, was due more to failure of the central nervous system rather than to any true immunity, as at the same time there was a failure to respond to inspired CO<sub>2</sub>-air mixture.

As/



As the observations pointed to central rather than peripheral action of the pressor extracts he next carried out a series of observations to determine at which level of the central nervous system the response was mediated. He found that in animals with the spinal cord pithed there was no pressor response. Section of the brain in anaesthetised vagotomised cats produced no change until the level of transection passed through the brain somewhat above the bony tentorium. At this level the responsiveness of the animal was slightly increased. However, section just below the tentorium abolished all the effects of the extracts.

The effects of the extracts on non-anaesthetised cats were observed. Pressure was observed by the method of Koch and Mies, and it was found that in general, pressure extracts gave a response some 5 - 10 mm. Hg. greater than the same extracts tested in the same animal while anaesthetised with ether.

Ultra-filtrates of plasma were tested on non-anaesthetised animals. Neither with normal plasma nor with plasma of malignant hypertensive nephritics were any pressor responses obtained. Furthermore, these ultra-filtrates were equally negative on anaesthetised/

anaesthetised animals. Extracts of erythrocytes were found to be powerfully vaso-depressor.

Turning now to Page's observations on plasma extracts from clinical cases of hypertension it may be stated briefly that he found no correlation between the amount of pressor substance and the height of the blood pressure. That this portion of his work was done with extreme thoroughness may be judged from the fact that he performed 165 experiments and used blood from almost every variety of disease associated with hypertension, including four cases of pituitary basophilism. In several of those cases he examined the cerebrospinal fluid by similar methods. Here again he found that although cerebrospinal fluid contains just as much pressor substance as plasma there is no significant increase of this substance in hypertension. To summarise this most comprehensive contribution, it has been found that there is a widely distributed pressor substance present in human plasma, ascitic and cerebro-spinal fluids. Its chemical properties suggest those of an organic base. It can be extracted from water by chloroform. It does not pass through ultra-filters, and it is suggested that the substance is held in a bound/

bound state by the plasma colloids and is liberated on coagulation of the colloids by alcohol. Pharmacological assay of the substance shows that its properties conform to those of no known pressor substance. Its action appears to be through the central nervous system, and in this action the release of adrenalin plays no part. The splanchnic area appears to be the portion of the peripheral vascular bed principally concerned. Lastly, and most important of all, no evidence has been produced by the method employed that the amount of this pressor substance is increased in the blood or spinal fluid of patients with hypertension of varied pathogenesis.

Conclusions, somewhat similar to those of Page (1935), were reached by Leiter (1936). He found that the intravenous injection of the heparinised plasma of human blood into unanaesthetised dogs produced no significant changes in the mean blood pressure recorded by direct methods. The use of the rat as the test animal diminished the dilution of the injected plasma and made possible the demonstration of pressor effects in 44 per cent of heparinised plasmas. However, the distribution of the pressor effects/

effects of the plasmas among the different clinical groups was independent of the type of hypertension or, indeed, of the presence of hypertension. He considers that there is no satisfactory evidence in favour of the hypothesis that any common form of clinical hypertension is caused by the presence of pressor substances in the blood.

Several investigators have studied the relative amounts of pressor and depressor substances in the urine of normal and hypertensive subjects.

Lange (1933) and Felix (1933) stated that they found diminished amounts of a specific depressor substance in the blood and increased amounts in the urine of patients suffering from essential hypertension. They advanced the hypothesis that essential hypertension is accompanied by a lowering of the renal threshold for a specific depressor substance, thus allowing it to escape in the urine with a consequent overaction of the vasoconstrictor mechanism. Capps, Ferris, Taylor and Weiss (1935) have investigated the pressor and depressor effects of urinary extracts. They prepared extracts from the urine of each of twenty-five subjects, comprising seven with malignant hypertension, four with chronic nephritis and hypertension, six with essential hypertension/

hypertension, and eight normal subjects. In the preparation of the urine extracts twenty-four hour amounts of urine were collected on ice, and the pressor material adsorbed on a specially activated charcoal, by shaking mechanically for five hours. The amount of charcoal used varied between 4 and 6 Gms. per litre of urine. The charcoal was then filtered off by suction and the dried charcoal extracted in a Soxhlet apparatus, first with acetone and finally with 95 per cent alcohol. The extraction time was three hours. The resulting extracts were evaporated to dryness in vacuo at temperatures not exceeding 37 C., and the residue of each extract was suspended or dissolved in 15 cc. of normal saline. Extracts were stored on ice till used.

Cats were used to test the extracts. The animals were anaesthetised with sodium amytal or urethane. The animals were prepared by exposing both common carotid arteries and both vagus nerves and inserting a cannula into the trachea; the blood pressure was obtained by means of a femoral cannula. Heparin was used as an anticoagulant as it was found that sodium citrates tended to pass into the circulation where it caused a definite depression of sensitivity to the extracts. Urine extract was injected/



injected at a uniform rate into the femoral vein and washed into the circulation with 3 cc. of warm normal saline. In order to standardise the results 3 cc. of the extract, representing one-fifth of the original twenty-four hour volume of urine, was used for each injection. Each extract was tested in several animals in order to reduce errors due to individual variations in the animals. The carotid sinus "pressor" reflex was elicited frequently by occlusion of both common carotid arteries in order to compare its effect with that of the extract and also in an attempt to test the animal's vasomotor sensitivity. In all cases the injection of extracts was preceded by the injection of a similar volume of saline solution in order to determine the blood pressure effect due to blood volume changes alone.

The typical blood pressure response produced by the injection of urine extract consisted essentially in first a depressor and then a pressor effect. The depressor portion of the curve lasted from ten to thirty seconds and the pressor portion from three to thirty minutes. The magnitude of these two effects varied considerably in different experiments/

experiments. A pure depressor curve was frequently obtained; a pure pressor curve more rarely.

Extracts of the urine from a given patient, collected from day to day, generally gave the same type of blood pressure curve. However, marked variation in the daily excretion of pressor substance was occasionally noted. This variation did not seem to be directly related to the volume of urine. Capps et al further found no relationship between the magnitude of the pressor effect and hypertension of any type. In fact, they obtained rather more marked pressor responses with extracts of urines from normal subjects than with those of urines from patients with hypertension.

Experiments were then performed in order to try the nature of this pressor substance in the urine. As a depressor effect preceded the pressor curve it was suggested that the pressor effect was a non-specific overcompensation on the part of the test animal to the initial depression of the blood pressure. This was disproved by the fact that at times pure pressor effects were obtained and also by the fact that atropine abolished the initial depressor effect without influencing the pressor response/

response. The fact that atropine so abolished the depressor effect suggests that the depressor substances belong to the choline group of compounds. The response of the test animals to the urine extracts was compared with the response to pituitary extract but no relationship was found between them. Furthermore, it was found that the method of extraction employed completely destroyed the activity of pituitary extract. Repeated injections of adrenaline carried out during the course of many of the experiments demonstrated that there was no relationship between its action and that of the urine extract. They further concluded that the shape of the pressor curves obtained from the urine extracts resembled in many respects those obtained from drugs with a central vasomotor action. The animal's response to the urine extract was, therefore, compared with that to alpha-lobeleine. A close similarity between the two effects was noted.

The two important conclusions in this careful and satisfactory research were (1) "The pressor substance or substances in the urine of patients with hypertension of any type is not increased above the/

the normal. The blood pressure response of test animals to extracts of urines from normal and hypertensive patients was essentially the same.

(2) Although the exact nature of the pressor substance found in urine was not determined, we have presented experimental evidence that it is not epinephrine or pituitary, and that it is a rather stable water-soluble substance that acts centrally rather than on the peripheral nerve endings or on the vascular system."

Inconclusive as have been the investigations into the "pressor body" hypothesis it must be realised that it is quite possible that some circulating substance may be absorbed by the plain muscle of the arteriolar wall and may alter its tonicity, without the substance ever reaching a concentration in the blood sufficiently great to be detected by any of the methods yet employed. One must, therefore, preserve an open mind in this matter and avoid regarding the paucity of reliable positive evidence as an effective negation of the hypothesis.

R E F E R E N C E S.

- ANSELMINO & HOFFMANN: Arch. Gynak., 1931, 147, 597.
- ANSELMINO & HOFFMANN: Arch. Gynak., 1931, 147, 621.
- ANSELMINO & HOFFMANN: Arch. Gynak., 1931, 147, 604.
- ANSELMINO & HOFFMANN & KENNEDY: Edin. Med. Jour.,  
1931, 39, 376.
- BOHN: Ztschr. f. klin. Med., 1931-32, 119, 100, 403.
- BRIGHT, R.: Guy's Hosp. Report, 1836, 1, 338.
- BURN: Quart. Jour. of Exp. Pharmacy & Pharmacol.,  
1931, 4, 517.
- BRYOM & WILSON: Quart. Jour. Med., 1934, 3.n.s., 361.
- CAPPS, FERRIS, TAYLOR, WEISS: Arch. Int. Med., 1935,  
56, 864.
- CURTIS, MONCRIEFF & WRIGHT: Jour. Path. & Bact.,  
1927, 30, 55.
- CUSHING: Amer. Jour. Path., 1933, 9, 539.
- CUSHING & GOETSCH: Amer. Jour. Physiol., 1910-11,  
27, 60.
- DANZER, BRODY & MILES: Proc. Soc. Exper. Biol. &  
Med., 1925-26, 23, 454.
- DE WESSELOW & GRIFFITHS: Brit. Jour. Exp. Path.,  
1934, 15, 45.
- ELLIOT & NAZUM: Jour. Lab. & Clin. Med., 1932-33,  
18, 1255.
- FELIX, K.: Klin. Wchnschr., 1933, 12, 176.
- GHEORGHIAN & NICULESCU: Compt. rend Soc. de Biol.,  
1924, 90, 1163.
- GREENWALD: Jour. Biol. Chem., 1924, 59, 329.
- HERRING: Quart. Jour. Exper. Physiol., 1908, 1, 281.
- HOSKINS & McCLURE: Amer. Jour. Physiol., 1912, 30,  
192.
- HOSKINS/



- HOSKINS & McCLURE: Arch. Int. Med., 1912, 10, 343.
- HOYLE: Quart. Jour. Med., 1933, 2 n.s., 549.
- HULSE & FRANKE: Arch. f. exp. Path. & Pharmacol.,  
1929, 143, 257.
- HULSE & STRAUSS: Ztschr. f. d. ges. exp. Med.,  
1924, 39, 426.
- INGIER & SCHMORL: Deutsch. arch. f. klin. Med.,  
1911, 104, 262.
- JANEWAY & PARK: Jour. Exp. Med., 1912, 16, 541.
- KESTRANEK, MOLITOR & PICK: Biochem. Ztschr., 1915,  
164, 34.
- KESTRANEK, MOLITOR & PICK: Biochem. Ztschr., 1927,  
186, 130.
- LANGE, F.: Klin. Wchnschr., 1933, 12, 173.
- LANDAU : Ztschr. f. klin. Med., 1907, 64, 227.
- LEITER, L.: Arch. Int. Med., 1936, 57, 729.
- MAJOR: Amer. Jour. Med. Sci., 1925, 170, 228.
- MAJOR: Johns Hopkins Hosp. Bull., 1925, 36, 357.
- MAJOR: Arch. Int. Med., 1927, 40, 891.
- MARX & HEFKE: Klin. Wchnschr., 1933, 12, 1318.
- MEYER: Ztschr. f. Biol., 1906, 60, 395.
- NEUSSER: Nothnagels Spezielle Pathologie u.  
Therapie., Vienna, 1898, 18, 71.
- O'CONNOR: Arch. f. exper. Path. u. Pharmacol.,  
1912, 67, 195.
- OLIVER & SCHAFER: Jour. Physiol., 1895, 18, 231.
- PAGE: Jour. Exp. Med., 1935, 61, 67.
- PEARCE: Jour. Exp. Med., 1908, 10, 735.
- SCHLAYER/

- SCHLAYER: Deutsch. med. Wchnschr., 1907, 33, 1897.
- SCHUR & WIESIL: Deutsch. med. Wchnschr., 1907, 33,  
1897.
- STEWART: Jour. Exp. Med., 1911, 14, 377.
- STEWART: Jour. Exp. Med., 1912, 15, 547.
- STEWART & HARVEY: Jour. Exp. Med., 1912, 16, 103.
- STEWART & ZUCKER: Jour. Exp. Med., 1913, 17, 152.
- THOMAS: Beit. z. path. Anat. u.z. allg. Path.,  
1910, 49, 228.
- VAQUEZ: Proc. Congre. Francais de Med., 1904, 338.
- VOLHARD: Verhardl. deutsch. Gisellsch. f. ind. Med.,  
1931, 77, 247.
- WHITE: Jour. Biol. Chem., 1927, 71, 419.
- WILSON & HOLIDAY: Biochem. Jour., 1933, 27, 1095.
-